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Abstracts

Posterbeiträge

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A photocleavable MeCAT-reagent

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Keywords: Proteomics, metal labeling, lanthanide DOTA, photocleavable, MALDI-Imaging

Einleitung
As part of proteomics, the need for reliable protein quantification methods steadily increases. Thereby, the use of stable isotopes for protein and peptide labeling including ICAT and iTRAQ are increasingly applied. Labels are based on a chemical labeling reaction with a specific reagent. Mass differences of differently labeled samples are then detected by mass spectrometry (MS) to gain qualitative as well as quantitative information. As a further quantification methodology, we developed MeCAT (Metal Coded Affinity Tagging) [1 - 2]. MeCAT uses chelate complexes of lanthanides for relative and absolute quantification. For the later, elemental mass spectrometry can be employed. Here, we introduce a photocleavable MeCAT-IA reagent, carrying an iodoacetamide moiety [2].

Experimenteller Teil
A new photocleavable linker including the MeCAT-reagent was synthesized. Proteolysed model proteins were labeled with this photocleavable MeCAT-reagent that contains a cysteine-reactive group for quantitative labeling, an elemental tag, loaded with a lanthanide ion for quantification and a photocleavable group for formation of characteristic fragments. The later can be used for quantification. For separating labeled peptides from other sample components, a HPLC was used coupled online to electrospray ionization (ESI)-MS. Furthermore, irradiation experiments with labeled peptides were performed, using an UV-lamp with the wavelength 366 nm.

Ergebnisse und Diskussion
The investigated proteolysed model proteins carry at least five cysteine residues in its sequence. The completeness of the labeling reactions was examined by employing cysteine containing peptides. The obtained spectra of labeled peptides from HPLC/ESI-MS were compared with the native peptide spectra. No unlabeled peptides were found after the labeling reaction with the photocleavable MeCAT-reagent. This was shown for all investigated model proteins. Hence, an important requirement for reliable quantification was fulfilled. In further experiments, the photocleavability of the new MeCAT-reagent was investigated. For that reason, an UV-lamp was used irradiating the labeled peptides, followed by HPLC/ESI-MS. A characteristic photo-fragment, which can be used for quantification in prospective experiments, was detected. Furthermore, the general fragmentation behavior of labeled peptides was investigated. Here, the fragmentation techniques CID and IRMPD were applied. In order to increase the analytical options, labeled peptides were also investigated by MALDI-MS and MALDI-Imaging.

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Posternummer: 2

A proteomic workflow for characterization of human skin biopsies by using pico-second infrared laser (PIRL)

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Keywords: Proteomics, DIVE, PIRL, tissue ablation, tandem mass spectrometry.

Einleitung

Human skin is the largest organ of integumentary system that protect the body from external environment and prevent excessive fluid loss [1]. Scientists are closer than ever to understanding the proteomes of skin [2]. However, the protein compositions in each type of cell layers, especially in epidermis, are not accurately defined. For further characterization of protein composition in layers of human skin we have undertaken a proteomic approach by using an innovative, “one-step” extraction and homogenization method, called DIVE (desorption by impulsive excitation of intramolecular vibrational states of water molecules in the cell), induced by irradiating tissue with a pico-second infrared laser (PIRL) [3]. The aim of this study was to test if with DIVE epidermis and dermis can be ablated separately.

Experimenteller Teil

The surfaces of frozen skin biopsies were ablated by PIRL at different laser energies. The collected proteins were digested with trypsin. Tryptic digested peptides were subjected to a nano-UPLC-ESI-MS/MS using a hybrid orbitrap system (Orbitrap-Fusion, Thermo Fisher scientific). Data analysis and protein identification was performed with Proteome Discoverer (Thermo Fisher scientific) using the Uniprot human protein database. For identifying epidermis markers respectively dermis markers, their protein lists derived from LC-MS/MS analysis and protein identification were compared. Candidate markers were validated by looking in the “The Human Protein Atlas” for the appearance of the candidate proteins in skin tissue sections stained by immunohistochemical methods.

Ergebnisse und Diskussion

As a first step in this study protein markers for epidermis and dermis were determined. From skin tissue applying a laser energy at 180 µJ per pulse, we ablated 24 µm of the skin tissue and identified in the resulting condensed DIVE ablation plume approximately 200 proteins (≥ 2 PSMs: number of fragment spectra of a defined unique peptide). 70 of these proteins were epidermis proteins regarding to protein marker database made by our group and 7 dermis proteins. With a laser energy of 160 µJ per pulse 22 µm tissue was ablated and 189 proteins, thereof 56 were epidermis proteins and four dermis proteins. With the lowest adjusted laser energy at 140 µJ per pulse, we achieved a deepness of 18 µm and identified 132 proteins, thereof 47 were epidermis marker proteins 2 dermis proteins). In summary, these results show that with the lowest laser energy we were able to ablate predominantly cells of epidermis.

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Posternummer: 3

A routine QC method to monitor high-level LC and MS performances on complex protein digests

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Keywords: UHR O-TOF

Einleitung

Generation of high quality proteomics data implies the analysis of several 10,000s analytes within a complex mixture, and can only be achieved if the LC-MS/MS platform delivers sustainably optimum performance. The regular use of LC and MS QC tests is therefore required as early indicators for system maintenance.

As label-free quantification techniques are getting more popular, the stability and reproducibility of retention times and signal intensities in LC/MSis crucial. They need therefore to be monitored, to guarantee the obtention of accurate quantitative results.

We present here a study showing inter-lab reproducibility nanoLC-MS performance monitored in a highly complex sample.

Experimenteller Teil

A ready-to-use predigested human cell line (K562, Promega Madison, USA) was used for QC, dissolved in 0.1% TFA to a concentration of 100 ng/µl. Tryptic peptides were separated on a nanoLC system (55 min gradient 2%-35% 0.1% formic acid in acetonitrile). Data was acquired on an impact II (Bruker, Bremen, Germany) equipped with a CaptiveSpray ionization source. Instrument was operated with 2 Hz MS and 4 – 16 Hz MS/MS acquisition speed, dynamically adapted to the precursor intensity. Database search was done using ProteinScape 3.1 (Bruker) equipped with Mascot 2.4 (MatrixScience, London, UK).

Ergebnisse und Diskussion

To adequately validate mass spec performance, complex biological samples are required.

We use a commercially available sample, consisting of a pre-digested human cell line, as this allows inter-laboratory comparison independently of sample preparation skills. A fixed instrument setup and corresponding methods are used, allowing in-depth comparison of nanoLC and MS performance. Usage of CaptiveSpray ionization source enables easy-to-use system setup also for non-experienced users, as complicated xyz-positioning of the nanospray source is not necessary. This further reduces user skills dependent results. The methods applied were selected based on their usability in routine workflows. Limited sample amount of 200 ng was injected to avoid necessity of several blank injections. Relatively short gradient of 50 min ensures only minor time interference with longer sample batches.

Monitored parameters are retention time, mass accuracy, and peak intensity for a predefined set of peptides as well as overall number of identified proteins and peptides. First results have been obtained in 3 different labs with 3 different users. High reproducibility of identification results was achieved, showing only ±6.5% variation of protein identifications and ±16% variation of peptide identification. This clearly displays feasibility of performing inter-laboratory comparisons using the selected setup. The study will be expanded to further labs. Comparison of obtained data will allow us to discuss usability of different quality control parameter for monitoring instrument performance.

Referenzen

A simple and cost efficient way to ship biological material

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Keywords: Quality control, Proteomics, Interlab Study

Einleitung
When conducting experiments in different laboratories across one or several countries it is of utmost importance, that the quality of the sample is not compromised by transport. In addition import regulations of other countries can cause problems and increase the costs. Sending frozen tissue samples requires more planning and administrative work than simply sending lyophilized peptides. The aim of this study was to test the impact of the transport, lasting several days in summer, without cooling the samples, consisting of dried tryptic peptides from tissue protein extracts.

Experimenteller Teil
20 5µm frozen tissue sections were digested. In short, tissue sections were moistened and transferred to a tube containing 300 µL 100 mM DTT in 100 mM AmBiCa and incubated for 10 min at 56 °C. Alkylation was done using IAA and trypsin was used as protease. After incubation over night the peptides were desalted using reversed-phase columns (Waters Oasis cartridges). The dried peptides were dissolved in 0.1% FA for LC-MS/MS measurement. An aliquot of the sample was dried in a vacuum centrifuge and send to Portugal using public transport without cooling and sent back to the lab using conventional air-mail. The dried tryptic peptides and the control sample were measured on the same day on an Orbitrap mass spectrometer.

Ergebnisse und Diskussion
There are no distinct differences observable in the MS1 chromatogram, which under these harsh conditions (the sample was delayed 10 days because of a strike and kept at the post office without cooling) demonstrates that at least most of the abundant peptides were not decreased in their amounts. To evaluate the sample on protein and peptide level MaxQuant was used to determine the number of protein IDs and peptide IDs in each sample. 90% of the proteins were identified in both samples on peptide level 85% of the same peptides were identified, which might be due to the intrinsic variance of DDA measurements. Even though there are less peptides identified in the shipped sample there are also some peptides which were only identified in this sample compared to the control. This enforces the possibility that these differences are in fact due to the intrinsic variance.

Referenzen

Posternummer: 5

Analysis and quantification of ADP-ribosylated Rho GTPases by mass spectrometry

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Keywords: ADP-ribosylation, C3bot, SILAC

Einleitung

Mono-ADP-ribosylation is a prevalent action of virulent bacteria to activate or inactivate host target proteins. C3 exoenzyme of Clostridium botulinum (C3bot) is a 24 kDa single-chain protein, which ADP-ribosylates small Rho GTPases leading to their functional inactivation. As C3bot lacks a binding and translocation domain, the cellular uptake is not well understood. To assess the uptake into target cells, the amount of ADP-ribosylated small GTPases RhoA and RhoC was determined by LC-MS. Furthermore, C3bot exhibits an axon and dendrite growth promoting function in primary hippocampal neurons. A quantitative proteomics approach revealed novel insights of C3bot treated immortalized hippocampal cells.

Experimenteller Teil

We created a heavy SILAC spike-in standard which consists of comparable amounts of ADP-ribosylated and non-modified Rho GTPases to characterize the C3bot uptake into hippocampal HT22 cells. Samples to be analyzed were cultivated in light SILAC media and were treated with C3bot. Cells were lysed followed by the combination with the heavy labeled spike-in standard. Proteins were digested with trypsin followed by LC-MS analysis. Target peptides were set on an inclusion list for privileged fragmentation.

Shotgun proteomics was performed with a SILAC triplex approach followed by trypsin digestion and LC-LTQ orbitrap Velos-MS (Thermo Fisher Scientific, Germany). MS raw data were processed with the MaxQuant software package. Regulation factors of selected proteins were verified by multiple reaction monitoring (MRM).

Ergebnisse und Diskussion

Evaluation of Rho ADP-ribosylation kinetics revealed a rapid modification of RhoA. More than 90 % of RhoA was modified after an incubation time of 1 h increasing to total ADP-ribosylation after 30 h incubation with C3bot. In contrast, the ADP-ribosylation of RhoC was delayed and only 40 % were modified after 1 h. The amount of ADP-ribosylated RhoC is increased to almost 90 % after 30 h. The slower modification rate of RhoC was either caused by substrate specificity or cellular compartimentalisation of RhoC in target cells. The established method enables the exact determination of modified and unmodified Rho GTPases, which allows the investigation and characterization of uptake mechanism of C3bot in target cells.

The shotgun proteomics approach revealed that almost 20 % of quantified proteins showed a significant altered abundance after an incubation of six days. Upregulated proteins were involved in signal transduction and cytoskeleton regulation, which corresponded to strong morphological changes after C3bot incubation. Moreover, nuclear proteins which are involved in transcription and ribosome biogenesis showed a reduced abundance. This quantitative SILAC approach revealed several protein groups which contribute to the alterations of hippocampal cells due to secondary or tertiary effect of C3bot treatment.

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Analysis of cerebrospinal fluid using high resolution mass spectrometry

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Keywords: label-free, quantification, workflow, cerebrospinal fluid, high resolution

Einleitung
Nowadays, the most common neurodegenerative disorders are Alzheimer's and Parkinson's disease (AD, PD). Although these disorders are extensively investigated, an exact diagnosis can only be confirmed post-mortem. To facilitate therapeutic intervention, an early diagnosis as well as the possibility to monitor disease progression is important. Since cerebrospinal fluid (CSF) is the body fluid that surrounds the brain it is a promising source for biomarker discovery of neurodegenerative disorders. For this reason multiple proteomic studies of CSF were performed to investigate differences between healthy individuals and patients suffering from neurodegenerative diseases. Here we show a label-free mass spectrometry (MS) approach to analyze CSF, which provides the great advantage of fast and cheap sample preparation compared to stable-isotope labelling methods.

Experimenteller Teil
In order to reach a deeper coverage of the CSF proteome and to enable the detection of low abundant proteins we investigated several alternative strategies to set up a robust label-free quantification method. In detail we investigated the impact of sample preparation (native CSF, depletion, solid phase extraction), tryptic digestion (in-gel versus in-solution) and HPLC conditions for peptide separation. The optimized method was subsequently used for a label-free quantification study. Within this screening differences at the protein level between PD patients and healthy individuals were examined in order to identify potential biomarkers. The samples were analyzed on a nanoHPLC coupled to a Q Exactive mass spectrometer.

Ergebnisse und Diskussion
The final label-free workflow consists of an in-solution tryptic digestion, determination of protein concentration followed by LC-MS data acquisition and data analysis/evaluation. By applying a strict FDR (false discovery rate) using a target-decoy database all identifications with FDR > 0.01 were discarded. Extensive analyses lead to the identification of over 5000 peptides corresponding to over 900 protein groups. Further data analysis was performed by following a combined intensity based quantification method using Progenesis® QI software and MaxQuant. In total 100 differentially expressed proteins were identified with a p-value < 0.05. Afterwards, a systematic selection of the differentially expressed proteins was performed. Using a bioinformatics strategy based on the ensemble feature selection method a panel of 10 proteins showed an average accuracy of over 80 %. These protein candidates will be validated for their suitability as biomarkers using an independent targeted mass spectrometric method.

Referenzen

Analysis of labile peptide phosphorylations by ETD mass spectrometry

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Keywords: Labile phosphorylation, EThcD, phosphate transfer

Einleitung
Mass spectrometry has become the technique of choice to identify phosphorylations of proteins. However, the reliable assignment of the modification site by tandem mass spectrometry (MS/MS) represents one of the most challenging tasks in phosphoproteomic studies. Phosphate-related neutral losses and gas-phase rearrangements which have been observed during collision-induced dissociation (CID) may prevent an unambiguous identification of the phosphorylation site, in particular in the analysis of extremely labile N-phosphorylations. Radical-driven electron-capture dissociation (ECD) or electron-transfer dissociation (ETD) fragmentation techniques have been described as an alternative for the analysis of modifications such as O-phosphorylations or glycosylation. Here, we report a study about the analysis of very labile N- and S-phosphorylations using ETD and ETD with supplemental activation.

Experimenteller Teil
Phosphorylated-lysine peptides were site-specifically synthesized by solid phase peptide synthesis using the chemoselective Staudinger-phosphite reaction. Peptides were analyzed by reversed phase nanoLC (Dionex Ultimate 3000 NCS-3500RS Nano) ESI-MS/MS (Orbitrap Fusion, Thermo Scientific). Triply and quadruply charged phosphor-peptides were fragmented using ETD and supplemental higher energy collisional activation (HCD). Precursor ions were isolated with the quadrupol using an isolation window of m/z 1.6 and captured in the iontrap with an AGC target of 1e5. After the ETD process ions were additionally fragmented in the routing pole using a normalized collision energy of 25%. FTMS2 spectra were measured in the orbitrap with a resolution of 15000.

Ergebnisse und Diskussion
Our data show that ETD fragmentation techniques overcome the limitations of collision-induced fragmentation in the analysis of very labile peptide and protein phosphorylations. Modifications which show complete neutral losses in collision-based fragmentation and which are not stable in solution under acidic conditions such as phospholysine and phosphocysteine are stable during ETD-MS/MS allowing an unambiguous assignment of the site of modification. However, as described for CID fragmentation of O-phosphorylated peptides during ETD process of N-phosphorylated peptides, neutral loss of phosphoric acid and phosphate transfer to other phosphor acceptors is observable. Depending on the peptide sequence and charge-state of the precursor ions gas-phase rearrangement, which would lead to false positive results in phosphoproteomic studies, has been monitored. To avoid “phosphate scrambling” and to facilitate unambiguous phosphorylation site localization, proper experimental conditions considering the proton mobility have to be applied. In addition, supplemental HCD activation increases the fragment ion yield of ETD spectra without losing the very labile phosphorylation sites. In EThcD, especially fragment ions with low mass to charge ratio increase in their relative abundance, which leads to higher a coverage of the peptide sequence and hence to a more reliable determination of phosphorylated-lysine peptides.

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Application of Microfluidic/Tandem Quadrupole LC-MS/MS for MRM Based Translational Research

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Keywords: IonKey, Xevo TQ-S, Synapt G2Si, Biomarker, Heart failure

Einleitung

The application of tandem quadrupole MS with microfluidic chromatography for the analysis of proteolytic peptides in human plasma is compared and contrasted with nanoscale LC and high-resolution oa-Q-ToF MS. A tandem quadrupole platform was considered for its performance in terms of sensitivity, selectivity, precision, and linearity. Microfluidic chromatography afforded the optimum balance of sensitivity and throughput, providing an ideal LC-MS configuration for the application to large sample cohorts. We also demonstrate that proteolytically digested, non-depleted plasma samples from heart failure patients could be classified with good discriminative power using a subset of proteins previously suggested as candidate biomarkers for cardiovascular diseases.

Experimenteller Teil

Various stable isotope labeled (SIL) peptides whose light analogues are putative biomarkers for cardiovascular disease (CVD) were spiked at various levels into un-fractionated, tryptically digested EDTA human serum. The SIL peptides were simultaneously spiked into diluted digested matrix (200 ng/uL) at 12.5 fmol/µl and serially diluted in matrix to various levels over the range 0.00625 - 12.5 fmol/ µl. Samples were injected, separated and detected using a reversed phase gradient on various LC-MS platforms. This analysis was replicated eight times with MRM acquisition modes using all combinations of IonKey/MS integrated microfluidics or a nanoscale LC system in combination with Xevo TQ-S, Xevo TQ-S micro, Xevo G2-XS QTof or SYNAPT G2-S.

Ergebnisse und Diskussion

Experiment wide evaluation was conducted by normalizing transitions intensities to the most abundant transition for a given peptide. Similar experiments were conducted for all possible configurations and only those transitions retained that illustrated good agreement. The concentration and coefficient of variation (CV) were calculated for each individual SIL spike-level, representing a multi-level single point average and error estimate. The results provide an estimate of MS to the experimental variation, where uncorrected CV values ranged from 10 to 30%. Internal standard correction reduced this 5 to 8%. Retention reproducibility was typically better than 1%. A throughput increase from the use of microfluidics was achieved, with an average 2-fold reduction in analysis time observed without a substantial in increase in the number of de-tected isobaric interferences. Multivariate analysis of proteins showed that patient samples could be classified using OPLS-DA. Using the data and results related to one of the SIL spike levels, partial separation of healthy controls and HF (combined HFPEF and HFREF) could be observed. The proteins contributing mostly to the separation were ApoA1, CRP and plasma protease C1 inhibitor.

In Summary, the IonKey/MS microfluidic platform affords twice the throughput over classical nanoscale LC. The Sensitivity in terms of S/N ratio was shown to be roughly comparable across the four MS platforms. The best combination of throughput, sensitivity, linearity and reproducibility was afforded by the IonKey/MS - Xevo TQ-S platform. Multivariate analysis showed that HF samples could be classified using OPLS-DA with near complete separation of healthy controls and HF patient samples.

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Posternummer: 9

Application of proteomics on bone cells in response to Mg-alloys compared to Ti- Implants

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Keywords: Magnesium, Implant, Titanium, bone, Proteomics, mass spectrometry

Einleitung

Bio-absorbable magnesium (Mg)-based implants display various advantages for bone healing via the opportunity of getting degraded by corrosion and the possibility of new bone formation via possible osteo-inductive properties (1, 2). Because of avoiding removal surgery, Mg-based implants can be more desirable than conventional permanent metal implants (3, 4).The aim of this study was to investigate how Mg-implants affect bone cells compared to conventional permanent titanium-implant materials. Therefore, we studied the effect of elevated Mg^{2+} compared to Ti^{3+} levels on osteoblasts by proteomics.

Experimenteller Teil

Proteomes of cultured osteoblast were investigated in absence (control) and presence of Mg-Implants after 7 days incubation and Ti-Implants with the same incubation time. First, cell pellets were lysed; proteins of the cells were extracted, reduced, alkylated and incubated with trypsin. All the samples were desalted before measurement by mass spectrometry. Then, desalted samples were subjected to a nano-UPLC-column coupled to a hybrid orbitrap system (Orbitrap-Fusion, Thermo Fisher scientific). Data analysis and data interpretation for comparison of relative protein abundance was performed with Proteome Discoverer (2.0) by using spectral counting and MaxQuant (1.5.2.8) based on precursor ion intensity.

Ergebnisse und Diskussion

More than 3500 proteins including more than 89,000 peptides were identified, and 135,000 spectra were recorded in total. 96 of the identified proteins have direct or indirect role in bone formation- or bone resorption, and the level of 41 of these bone formation/resorption proteins were changed after exposure of Mg^{2+} or Ti^{3+}.

The investigation shows that Mg-implants are supporting bone healing process. For instance, the level of Matrix metalloproteinase-2, which is involved in tissue remodelling and deficiency of it leads to reduced bone growth, bone density and quality, required for osteoblastogenesis, was increased in osteoblasts through rising the incubation time with Mg-Implant, much higher than Ti-Implant and control cells.

Our results confirm that both Mg^{2+} has an effect on protein composition in bone cells. We observed the composition of osteogenesis-related proteins changed more significantly in the presence of Mg^{2+} compared to Ti^{3+}. Whether this suggests that Mg affects bone formation more strongly than Ti has to be addressed in future experiments.

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Posternummer: 10

Applying a Proteoform profiling method for neurological disorder biomarker discovery

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Keywords: Protein profiling, UHR-QTOF MS, neurology, Top-down proteomics

Einleitung

Measuring the intact mass of proteins in tissue samples or biofluids has the advantage over bottom-up approaches to be able to reveal much more easily the results of major biological processes like alternative splicing, proteolytic processing or modification of PTM pattern distribution, as the information relative to the different proteoforms distribution is encoded in the intact mass of proteins.

In this study we have used a last-generation UHRQ-Tof to perform a protein profiling approach with the objective of detecting and afterwards identifying proteoforms which are specific are discriminating for neurological disorders

Experimenteller Teil

Pool CSF samples obtained from control patients with ethical consent. 1.5 µl CSF samples were separated on a 75µmX15cm µm pepmap C4 column on Ultimate nano-RSLC system (TMO) after preconcentration on a monolithic pepmap C4 300µm x 5mm trap (TMOc). LC was coupled to an impact II QTof (Bruker) with CaptiveSpray ion source (Bruker) operated in MS and auto MS/MS modes.

All data was automatically processed (calibration, protein signal extraction with Dissect™, deconvolution and obtaining monoisotopic masses with SNAP™, export of deconvoluted monoisotopic masses with corresponding retention time and intensities). Statistical analyses were performed on a slightly modified version of the ProfileAnalysis 2.1 Software. Identifications were performed using Top-Down Sequencing search functionality of BioTools 3.2 (Bruker) and Mascot 2.4(Matrix Science).

Ergebnisse und Diskussion

Using High-Quality threshold protein detection, we could easily detect over 1500 proteoforms (from doubly charges peptides up to 35Kda proteins) with very high reproducibility (>.

The spectral quality observed for single compounds was preserved while measuring these highly complex mixtures: the highest mass shift for the monoisotopic peak of a glycosylated form of the fragment 228-339 for the human chromogranin-A (13,2 Kda) over 4 injections was 0,19 ppm, while the resolution was exceeding 45 000. Analysis of the first technical replicates enabled to detect from a small pool of biological replicates enabled to detect several discriminating proteins. An Auto-LC MS/MS run with a set up with the use of a Scheduled Precursor list enabled the identification of a glycoform of a human Chromogranin-A fragment. The study is now continued with samples issues from a larger cohort of patients in order to validate the early biomarker candidates already detected.

The last generation UHRQ-TOF, by combining a large spectral dynamic range to the capacity of preserving a high spectral quality over a large mass range in complex mixtures, are now capable of delivering rapidly a high-quality proteoform distribution information that has the potential to complement the information delivered by the bottom-up approaches.

We are now establishing the applicability of the approach for a study on real life clinical samples.

Referenzen

Artefactual modifications of unblocked reactive cysteines

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Keywords: artefactual cysteine modifications

Einleitung
Cysteine is one of the most abundant, yet most reactive amino acids [1]. It is thus susceptible to oxidative stress [2] and modifications during handling. Some cysteines may already be oxidized under slightly basic conditions, as is, e.g., the case during tryptic digestion. This process generates various cysteine modifications in an uncontrolled manner including S-sulfenic and S-sulfinic acids as well as S-sulfonation. Such modifications might be mistaken for PTMs in biological assays. They also introduce errors in quantification. In order to obtain a clearer picture of the reactions we have investigated the isolated terminal peptide LSFNPTQLEEQCHI from β-lactoglobulin during incubation in NH4HCO3-solution.

Experimenteller Teil
β-lactoglobulin (10 nmol, Sigma-Aldrich) was transferred to an Amicon filter unit (Millipore, 10 kDa cut-off), reduced with 10 mM TCEP (Sigma-Aldrich) for 1 h, washed four times with 100 µL of 50 mM NH4HCO3-solution (Fluka), and trypsinized on the filter for 14 min (Serva, protein-to-enzyme ratio 50:1). The peptides were collected by centrifugation and fractionated by solid phase extraction (ZipTips C18, Agilent Technologies) using solutions of 1% HCOOH (Fluka) with rising concentrations of acetonitrile (ACN, Fluka). The fraction containing 62 % ACN was evaporated to dryness, resuspended in 100 µL of 50 mM NH4HCO3-solution (pH 8.4) and incubated at 37 °C for 14 h.

PseudoMRM using RP-LC-MS/MS was performed at different points of time (nanoAcquity, Q-TOF Premier, Waters Corp.).

Ergebnisse und Diskussion
Additional peaks appeared in the chromatogram resulting from modifications by -354 Da (loss of CHI fragment), +25 Da (S-cyanylation), +32 Da (S-thiolation), and +80 Da (S-sulfonation). The amidated dimer was also observed. Co-eluting species represented modifications by -34 Da (dehydroalanine), +48 Da (S-sulfenylation) and +64 Da (S-sulfination). After 1.5 h, the intensity of the unmodified peptide LSFNPTQLEEQCHI was already reduced by more than half. At the end of the incubation period only traces of the unmodified peptide could be detected. Instead, the most prominent peaks resulted from dimerized peptide and peptide modified by S-thiolation, S-sulfonation and by the loss of the CHI fragment.

Although we found only few cysteines behaving as extreme as the cysteine in the examined peptide, routinely inclusion of an alkylation step in protein work-up protocols is advised to prevent artefactual cysteine modifications. This is crucial, e.g., when searching for phosphorylation sites as the mass difference to S-sulfonation is only 9,5 mDa and these modifications may easily be confused.

In order to prevent possible overalkylation [3] the reaction should be stopped either by removing the alkylating reagent or by quenching with DTT. In case S-alkylation absolutely has to be avoided, the reducing agent DTT could be added to all solutions. This measure, however, may reduce trypsin activity.

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Balancing Speed, Sample Complexity and Data Quality in Proteomics Applications on a benchtop Quadrupole-Orbitrap mass spectrometer

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Keywords: Peptide ID, Protein ID, Orbitrap

Einleitung
Understanding the role of proteins in different states of the body has significantly influenced innovation in proteomics based methodologies. Shotgun proteomics is one of the most commonly used techniques and is based on proteolytic digestion of proteins, resulting in peptides that are subsequently separated by liquid chromatography and analyzed by tandem mass spectrometry followed by bioinformatics interpretation [1]. While the LC-MS method setup has become mature and user-friendly, continuous user involvement is needed to improve and optimize all parts of such workflow for individual samples of high complexity. Optimized settings to maximize protein identifications in shotgun analysis are discussed.

Experimenteller Teil
Lyophilized proteolytic HeLa lysate was re-suspended in 2 % ACN/0.1 % TFA. A dilution series from 0.1ng – 1 ug was prepared from this aliquot and separated with a ThermoScientific™ EASY-nLC 1000™ nano-HPLC system using 30 and 60 min gradients. Thegradient was later increased to 120 and 150 min for 1 - 4 ug of sample load. The eluting peptides were analyzed on the Thermo ScientificTM Q ExactiveTM HF mass spectrometer in data-dependent TopN acquisition mode. The acquired raw files were processed using Thermo Scientific Proteome Discoverer™2.0 software with the SEQUEST HT® search algorithm. Proteins and peptides were identified applying a false discovery rate of 1%.

Ergebnisse und Diskussion
Speed of acquisition and data quality play an important role in mass spectrometric based proteomics experiments. Data quality can be assessed by the success rate, i.e., the ratio of identified to measured spectra. However, success rate can be influenced by the database search parameters, gradient length, MS2 data quality, ion accumulation time and carryover from previous samples.

Keeping other MS parameters constant, the easiest way maintaining data quality for various sample loads at a given gradient length is to correctly set the ion accumulation time (maximum injection time) for MS2 scans in the method. As a rule of thumb, higher sample loads and shorter LC gradients will benefit from shorter maximum injection times. For different sample loads investigated with 30 min gradient length, between 150 and 3400 grouped proteins were identified. Database search results deriving from 30 or 60 min gradients show minimal differences in number of identified proteins and unique peptides for low sample loads up to 10 ng. The results also show that low sample loads do not really benefit from long gradients. Furthermore, our results suggest the following approach: While longer MS2 ion accumulation times could potentially provide improved success rates, the total number of MS2 spectra and hence the number of identifications potentially drops. Too short injection times on the other hand will result in low success rate; this is rationalized by a growing number of MS2 spectra of poor quality.

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Posternummer: 13

Characterization of carbohydrate binding sites in galectins by proteolytic affinity - mass spectrometry

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Keywords: Galectin-3, affinity interaction, proteolytic extraction, mass spectrometry

Einleitung

Galectins are a family of proteins defined by their binding specificity for β-galactose-containing glycoconjugates and share primary structural homology in their carbohydrate-recognition domains (CRDs). They are involved in crucial cellular processes such as cell-cell adhesion, cell migration, cell development and differentiation, chemotaxis and apoptosis. Together with another class of animal lectins, the C-type lectins, they exert various functions within innate immunity mechanisms. Beyond being involved in crucial cellular processes, galactosides inspired new lead compounds for glycan-directed drug design can be derived from lectins. The elucidation and availability of peptides mimicking the target specificity of the galectin would enable tests for clinical applicability, either to elicit favourable effects such as antitumor signalling or interfere with premalignant processes.

Experimenteller Teil

Proteolytic extraction mass-spectrometry was used to identify the ligand-contacting peptides. Proteolytic digestion of galectin-3 was performed with a trypsin/galectin ratio of 1:100 and the proteolytic mixture bound to the affinity matrix (lactosylated Sepharose 4B) in phosphate-buffered saline. Unbound material was then removed by thorough washing with buffer to ensure that even weakly bound material would be removed, prior to competitive elution with buffer solution containing 0.3 M lactose. The eluted epitope fraction was collected, desalted and characterized by ESI-IonTrap mass spectrometry.

Ergebnisse und Diskussion

The mass spectrometric analysis of the last washing faction yielded no peptides, and analysis of the elution fraction yielded two distinct tryptic peptides. Together, these two peptides harbored the key amino acids in contact with the ligand, thus representing the bioactive sequence parts of galectin-3.

This method allowed rapid molecular determination of carbohydrate binding sites in galectins with high sensitivity and low requirements in sample purity.

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Comparative assessment of 2DLC and GelfreeLC separation of the proteome of M. mazei

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Keywords: Model organism, Proteomics, LC-MS, Gelfree separation

Einleitung
Archaea represent the second domain of Prokarya and despite their close physical resemblance to bacteria, they are phyolgenetically more closely related to eukarya. Carbon and energy metabolism of the model archeaeon Methanosarcina mazei has been well characterized. Interestingly, unlike many methanogenic archaea which are limited to growth on H2 and CO2, M. mazei has a versatile metabolism capable of utilising a range of metabolites, such as H2 + CO2, acetate, methylated amines, and methanol. This metabolic versatility is mirrored in the comparatively large genome of M. mazei, which is considerably larger than the majority of methanoarchaeal species sequenced to date. This grants the organism a diverse tool box of genes and proteins, many of which remain to be functionally annotated.

Experimenteller Teil
We have utilized a combination of 2D-LC (high pH revered phase chromatographic fractionation followed by low pH ion pair reversed phase chromatography) and Gelfree-LC (GELFREE separation followed offline by low pH ion pair reversed phase chromatography) approaches, coupled to tandem MS analysis on a Thermo Q-Exactive plus mass spectrometer.

Ergebnisse und Diskussion
The methods utilized have allowed us to identify more than 2400 proteins of the predicted proteome of M. mazei. This represents the most comprehensive proteomic analysis of M. mazei to date. In addition, a comparative analysis of the various separation methods has been performed to provide an overview of the intrinsic biases present in each separation method. Furthermore, this analysis was able to tentatively identify a number of recently discovered small open reading frame (sORF) encoded proteins.

The accurate identification of a high percentage of the proteome of an organism is an essential starting point for future proteomic analyses. By determining the proteins that are translated during standard growth a benchmark is developed that will allow for future comparative analyses under alternative/challenging growth conditions. Furthermore, the identification of several newly identified sORF encoded proteins opens avenues of research into their role and function. In addition, understanding the intrinsic limitations of orthogonal separation schemes allows better tailoring of methods to specific samples.

Referenzen

Comparison of modern chromatographic material for protein separation in Top-Down Proteomics

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Keywords: Top-Down proteomics, protein separation, liquid chromatography

Einleitung

For today's top-down proteomics experiments an effective sample preparation and separation is needed to get the best results. Gelelectrophresis and liquid-chromatography are mainly used to clean up and separate complex samples before digestion. Using LC for separation of samples containing detergents the stability of the chromatographic material plays a major role for reproducibility of the results. Gorka et al. showed the benefit of chromatographic RP-material like POROS (Life Technologies GmbH, Darmstadt) getting good separation results of whole proteins, containing common detergents and impurities, prior to the digestion of cytochrome bc(1) complexes [1]. This work presents a comparison of the separation efficiency of different modern chromatographic materials for Top-Down Proteomic experiments.

Experimenteller Teil

POROS resin was used as reference material for comparison with Waters BEH 300 C4-(Waters GmbH, Eschborn) and Phenomenex' Aeris Widepore XB-C8-material (Phenomenex, Aschaffenburg). For each material a trap column and an analytical column were in-house packed by use of a packing pump (Sun Chrom, Friedrichsdorf) and untreated fused-silica capillaries. The trap columns had a dimension of 50mm x 0,15mm and the analytical columns of 200mm x 0,1mm. Measurements were performed on a Proxeon I NanoLC System (Proxeon Biosystems) combined with a SpectralFlow 501 UV-detector (Sun Chrom, Friedrichsdorf) with a 3nl flow cell installed. For data acquisition ChromStar 7 was used.

Ergebnisse und Diskussion

This investigation is part of a method development for analyzing complex protein samples via a top-down strategy with the aim to identify all containing proteins. As an alternative to SDS-Page the used materials should be robust against detergents and other impurities, which are often used in biochemical experiments to stabilize proteomic samples, while keeping a good separation quality and a maximum of peak capacity.

The biological protein sample (complex I of the respiratory chain from Yarrowia lipolytica) contained 41 proteins. The sample was stabilized with 100mM NaCl and a 20mM Tris buffer at pH 7.2. Polidocanol was used as a detergent in a common concentration of 0,025%. 1 pmol of the whole complex was separated over a 70 minute gradient elution followed by a 10 minute washing step. The flowrate was 500nl per minute. To evaluate the chromatograms in a simple way peak shape and peak capacity were inspected, considering that the peak capacity is the best measure of the performance of a gradient separation [2].

The chromatograms showed that all used chromatography resins are comparable or better than POROS material for the separation of intact proteins. Peak shape was better with the BEH material in comparison to the POROS resin. Peak capacity increased with the other materials. After separation proteins were free of salt, buffer or detergents and could be fractionated for further mass spectrometry experiments.

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Determination of N-glycosylation by i-HILIC based solid phase extraction for the analysis of intact glycopeptides by mass spectrometry.

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Keywords: glycopeptide enrichment, ZIC-HILIC, solid phase extraction

Einleitung

Glycosylation is one of the most frequently found posttranslational modifications (PTM). Glycosylated proteins are involved in various essential cellular processes, such as protein folding, receptor-ligand signalling or antibody-antigen recognition. However, determination of protein glycosylation is a challenging task owing to the tremendous heterogeneity of this PTM.

Yet, selective enrichment of proteolytic glycopeptides from complex mixtures by zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) followed by mass spectrometric analysis has been shown to yield almost complete structural information on glycan, peptide sequence, and glycosylation site [1,2].

Here, we present an alternative protocol aiming at N-glycopeptide enrichment based on the recently introduced i-HILIC stationary phase material.

Experimenteller Teil

Standard proteins were reduced, alkylated and submitted to enzymatic proteolysis. Glycosylated peptides were enriched by use of i-HILIC solid phase extraction (SPE) from the digests. Mass spectra and MS/MS experiments were obtained by direct infusion nanoESI Q-ToF mass spectrometry.

Ergebnisse und Diskussion

Owing to the low ionisation efficiency of glycopeptides and signal suppression by the vast majority of non-glycosylated peptides, the analysis of glycopeptides directly from proteolytic digests is often hampered by low intensity or even non-detectable signals. Furthermore, an elevated heterogeneity of glycan structures lowers the abundance of individual glycopeptide species. In order to avoid these complications tedious, laborate, and expensive HPLC MS procedures are frequently and successfully performed. These difficulties may be conquered in part by use of rather unspecific proteases that yield short, non-glycosylated peptides of low molecular weight and mainly glycopeptide-derived signals in the higher m/z range. However, overlaps of signals originating from glycopeptides and non-glycosylated peptides often demand a complete separation of the glycosylated species. This could be achieved by SPE using a novel HILIC stationary phase, viz. i-HILIC.

Commercially available glycoproteins were submitted to proteolytic digestion using both specific and unspecific proteases (trypsin, trypsin/chymotrypsin, thermolysin). Following selective enrichment by use of i-HILIC, N-glycopeptides were analysed by direct infusion nanoESI MS. One advantage of the latter “low-end” technique is the extended time frame for MS/MS experiments - compared to the typical chromatographic HPLC peak width - enabling e.g. multiple CID experiments and/or variations of collision energies. Preliminary data suggest that i-HILIC provides a similar selectivity for N-glycosylated peptides as observed for the well-established ZIC-HILIC material. Mass spectral data obtained in this study allowed for structural analysis of the glycan chain as well as part of the peptide backbone for identification of the attachment site.

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Posternummer: 17

Development of a generic proteomics method utilizing self-optimizing acquisition speed on a UHR-QTOF MS

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Keywords: Protein Identification

Einleitung
Shotgun proteomics aims for rapid identification and quantification of a large number of peptides and proteins from complex biological samples like whole cell lysates. The very high dynamic range of these samples remains the major challenge. Therefore high sequencing speed has to be complemented with high spectrum quality even for low abundant signals.

Here we investigate an automatic adaption of the MS/MS acquisition speed to the precursor intensity. With this, expert results can be achieved even for challenging samples without need for extensive method optimization. This is especially beneficial, if sample amount or analysis time is limited.

Experimenteller Teil
To investigate the performance of a generic proteomics method (InstantExpertiseTM) for different sample amounts, lysates of a human cancer cell line (HeLa) and of bacteria (E. coli) were digested and analyzed (5 ng - 5 µg). For quantitation experiments lysozyme C, myoglobin, ribonuclease and serum albumin were chemically labeled with iTRAQ in ratios of 10:10:5:5:2:2:1:1 and spiked into 200 ng of E.coli lysate. Samples were analyzed by nanoflow UHPLC (U3000, Thermo Scientific) online-coupled to an UHR-Q-TOF instrument (impact II, Bruker) using a CaptiveSpray ion source. Chromatographic separation was carried out using 50 cm columns (75µm PepMap Column, Thermo Scientific). ProteinScape 3.1 (Bruker) was used for peptide identification and quantitative analysis.

Ergebnisse und Diskussion
For large sample amounts (e.g. 5 µg of tryptic HeLa digest) we demonstrate thatdata analysis at maximum MS/MS acquisition speed is suitable and results inmore than 4,000 protein identifications during a 90 min gradient. Analysis oflower sample amounts (e.g. 5 ng of tryptic HeLa digest) yields less thanoptimal results when data are acquired with maximum acquisition speed andmethod development becomes crucial. The generic method instead ensuressuccessful peptide identification based on a balanced acquisition speed between4 - 16 Hz depending on the precursor intensity, allowing about 1,000 proteinidentifications from 5 ng HeLa digest. Preliminary quantitative data of 200 ng E. coli lysate spiked with iTRAQlabeled lysozyme C, myoglobin, ribonuclease and serum albumin were investigatedwith regard to the theoretical ratios of these proteins at 10:10:5:5:2:2:1:1. Areference amount of 4 ng was used, providing a quantification accuracy of allfour proteins at 10:10.2:5.5:5.4:3.3:3.5:2.1:2.8. Finally we introduce a novel approach to enable the generic proteomics method.Instead of defining the MS/MS acquisition speed based on the precursorintensity, e.g. 4 - 16 Hz, we reduced the method setup to a single parameter,the 'target intensity', and the system optimizes its operating conditions toachieve that intensity.

Referenzen

Efficiency of tissue homogenization via picosecond-infrared laser (PIRL) and classical homogenization as sample preparation step for proteomics

Autoren: Nimer, Refat (1); Kwiatkowski, Marcel (1); Hansen, Nils-Owe (2); Miller, R.J.Dwayne (2); Schlüter, Hartmut (1)

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Keywords: Picosecond Infrared laser (PIRL), desorption impulsive vibrational excitation (DIVE), Top-down proteomic

Einleitung

A novel method for ultrafast and soft cold vaporization of tissue via desorption by impulsive vibrational excitation (DIVE) using a picosecond-infrared laser (PIRL) has been developed by Dwayne Miller and his group [1]. By irradiating tissue with PIRL proteins are transferred from the tissue into the gas phase within at aerosol plume without changing the chemical formula of protein. The chemical composition of the protein is maintained during tissue ablation via PIRL. In this study, the efficiency of tissue homogenization performed with PIRL and with classical homogenization was investigated using two-dimensional electrophoresis (2DE) followed by mass spectrometric analysis (MS) of the tryptic peptides of the separated proteins from the 2DE gel.

Experimenteller Teil

Porcine muscle tissue was homogenized with DIVE according to Kwiatkowski et al. [2] or by a classical mechanical method (lyophilization of the tissue, grinding of the dried tissue, homogenization of the tissue powder in a extraction Buffer, centrifugation). Protein of both homogenates were separated 2DE. The assigned spots in both 2DE gels were cut and digested by trypsin. The resulting tryptic peptides were analysed by LC-MS / MS using Q-TOF MS and Orbitrap MS. Protein identification was performed by processing the MS data by Mascot (SwissProt, www.matrixscience.com) and Proteome-discoverer (2.0) searching against a mammalian protein database.

Ergebnisse und Diskussion

The assigned spots in 2DE from both DIVE and mechanical methods homogenates were analysed and revealed that more proteolysis products were present in the mechanical homogenates than in the DIVE homogenates. E.g. fructose-bisphosphate aldolase A protein was identified in 3 spots at lower molecular weight area (<25 kDa) in the 2DE-gel of the mechanical homogenate. Fructose-bisphosphate aldolase A species were absent at the same area in the 2DE-gel of the DIVE homogenate, In addition, a comparison between two 2DE patterns showed a decrease in protein species in the 2DE-gel of the mechanical homogenate, which indicated that diverse enzymatic activities during mechanical homogenization might be responsible for removing posttranslational modifications. Eg fructose-bisphosphate aldolase A (39.3 kDa) was identified in two spots in the 2DE-gel of the mechanical homogenate in comparison to 4 spots in the 2DE-gel of the DIVE homogenate. Because DIVE is very fast, the enzymatic conversion reactions are minimized. Thus, it can be concluded that extraction of proteins by DIVE is more effective than mechanical homogenization.

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Examination of Gas-phase Cationic Ions of Histidine Complexes via Infrared Multiple Photon Dissociation (IRMPD) Spectroscopy

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Keywords: IRMPD-spectroscopy, DFT-Calculation, gas-phase analysis of histidine complexes

Einleitung

Histidine rich sequences in proteins are frequently found in nature, e.g. in bacterial chaperones, in intrinsically disordered proteins (IDPs) and in poly-His taggs for immobilized metal affinity chromatography (IMAC) and are known to be responsible for multi-dentate binding of metal cations such as Cu2+ and Ni2+. However, the binding motifs of metal cations to peptides are complex and remain subject of extensive research efforts. Especially, the documented dependence of more than a single histidine moiety to generate stable nickel peptide-complexes is not well understood on a molecular level. To gain insight in the respective coordination-spheres of metal-His complexes we synthesized [M(His)(His-H)]+; [M(HisHis-H)]+ complex molecular ions with M= Ni2+, Ca2+ and probed these precursor ions with tandem MS, theory and IRMPD-spectroscopy.

Experimenteller Teil

A 3-D quadrupole ion trap mass spectrometer (QIT, Bruker Amazon Speed w/ETD) with an electrospray ionization (ESI) source, coupled to a free electron laser for infrared experiments (FELIX) was used to perform the infrared multiple photon dissociation (IRMPD) experiments.

The HisHis-dipeptide was custom-made in the group of Professor I. Neuendorf by A. Reinhardt. The gas-phase complex ions were generated by ESI from a solution of the respective sample in methanol. The IRMPD spectra were recorded by monitoring the intensity of the most abundant product ions and the depletion of the respective precursor ion as function of IR frequency over the 500–1800 cm-1 range. DFT calculation used B3LYP functionals with cc-pVDZ or 6-311g(d,p) basis set for Ni2+ or Ca2+,respectively.

Ergebnisse und Diskussion

Histidine complexes with Ni2+ were investigated as model compounds for (His)nNi2+ complexes. As a starting point for our studies we tried to synthesize and to characterize the mono-histidine-nickel complex [Ni(His-H)]+. However, all our efforts failed, highlighting the necessity of more than one His ligand to accommodate the demands of the divalent nickel cation to form a stable complex. To further investigate this we elucidated the [Ni(His)(His-H)]+ complex. Furthermore the HisHis-dipeptide was synthesised and the respective singly charged nickel complex [Ni(HisHis-H)]+ was probed by IRMPD-spectroscopy. Theory proposes three stable isomers with relative energies of 0 kJ/mol, 4.2 kJ/mol and 8.4 kJ/mol. Characteristically for all three computed [Ni(HisHis-H)]+ conformers is the deprotonated amide nitrogen of the peptide bond and the iminolate binding motif. This finding offers a plausible explanation for the instability of the [Ni(His-H)]+ complex as a peptide bond amide nitrogen is unavailable for deprotonation and coordinative binding. The overall convincing agreement between theory and experiment suggests the concomitant presence of the three conformers identified.

In comparison to that we also probed the complex of the HisHis-dipeptide with Ca2+. According to literature and in stark contrast to the nickel complexes of histidine the calcium cation adopts a sandwich complex binding motif with a deprotonated C-terminus.[1] The DFT computations clearly confirm this assumption. The fundamental studies of Armentrout et al.[2] on histidine and 4-phenyl-imidazole molecular ions as well as the contributions of Dunbar et al. on peptide bond tautomerization induced by divalent metal ions[3] serve as reliable benchmark-results.

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Exploring Transition from SWATH® Acquisition to MRM Analysis for Quantitative Proteomics

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Keywords: MRM, DIA, Quantitation, SWATH, Peptide

Einleitung

Targeted quantification is often desired to obtain accurate quantification in complex mixtures. Data independent acquisition combined with targeted data processing provides high quality quantitative results. The advantages of this approach are numerous: no assay development is required, data can be mined retrospectively, MS/MS based for good specificity. The data quality using this technique approaches that of MRM analysis on a triple quadrupole. However, to obtain good quantitative statistics for low abundant species, MRM approach is still the method of choice.

In this study, we compare quantitation workflows by transitioning a DIA method to a subset of targets using MRM analysis.

Experimenteller Teil

SWATH® acquisition of 500 ng of E. coli tryptic digest was performed using a QqTOF system interfaced to a nanoflow source and a chip based LC system. Replicate analysis was performed for this sample to obtain quantitation statistics. Data was processed using the PeakView® software and analyzed in Excel. Data for the protein/peptides of interest was then plugged into Skyline for generation and optimization of the MRM methods. 678 MRM transitions developed in Skyline were analyzed on a hybrid triple quadrupole ion trap system using time scheduled MRM. Replicate runs were obtained and all the quantitation processing was done using MultiQuant™ software.

Ergebnisse und Diskussion

Abundance of various E. coli proteins/peptides obtained from the SWATH® acquisition method was examined, intensity of data covered ~4orders dynamic range. From 5 replicates, XIC for 23,200 fragment ions corresponding to 4609 peptides and 999 proteins were processed. Of these fragment ion XICs, 88% showed CV's of ~ 20%. Subsequently, a number of proteins across the range in abundance were chosen from the data set for MRM analysis on the QQQ. Selection of fragment ions for MRM was based on the fragment intensity observed in the MS/MS spectra obtained from the QqTOF data. The fragmentation on both platforms was very similar; thus generation of MRM transitions was straightforward. Low intensity peptides were chosen to enable impact of selectivity and sensitivity on data quality. For low intensity proteins with only single peptides quantified, in silico MRM transitions of other peptides from the same protein were added to the assay, and targeted using MRM triggered MS/MS. This enabled the detection of more peptides due to the sensitivity improvement of the QTRAP® 6500 system over the TripleTOF® 5600 system. Final assay of 678 scheduled MRMs from 151 peptides (51 proteins) was run on 15 replicates. The %CV of 92% of these transitions were <20%. %CVs of the low abundant peptides obtained from DIA vs MRM were also compared and peak quality and %CV from the QQQ were improved. This work illustrates the advantage of combining DIA strategies for highest multiplexing with MRM analysis for highest sensitivity to provide a robust peptide quantification pipeline.

Referenzen

Full Validation of Therapeutic Antibody Sequences by Middle-Up Mass Measurements and Middle-Down Protein Sequencing

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Keywords: Biopharmaceutical characterization, antibody, middle-up/middle-down protein analysis

Einleitung
The regulatory agencies require comprehensive primary sequence validation for innovator and biosimilar monoclonal antibodies. Full sequence coverage is typically obtained by the combination of multiple LC-MS/MS datasets from various protease digests. In this work, we report a new middle-up LC/MS subunit analysis and middle-down LC/MALDI in-source decay method applied to cetuximab, panitumumab and natalizumab (representative FDA and EMA approved monoclonal antibodies). The goal was to unambiguously confirm their reference sequences and show the general applicability of the method. All antibody sequences were fully validated in this work by this combination of middle-up MW determination and middle-down (MD) protein sequencing.

Experimenteller Teil
The antibodies were obtained in their formulation buffers, deglycosylated with EndoS, and the Fc/2, Fd and LC domains were obtained by IdeS digestion and reduction. They were LC-separated and analyzed with an ultrahigh resolution QTOF instrumentation providing isotopic resolution of all mAb domains at ~25 kDa. Monoisotopic molecular weights were determined after Maximum Entropy deconvolution using the SNAP algorithm. LC/MALDI datasets were acquired to identify the relevant fractions, which were directly sequenced by MD on a high-resolution MALDI-TOF/TOF. MALDI-ISD spectra were compared to the respective sequences, and best matches reported for more detailed analysis.

Ergebnisse und Diskussion
The initial step to validate originator antibody sequences was the MW determination of the mAb domains Fc/2, Fd and LC. All domain MWs matched their respective sequences with an average mass accuracy of 0.67 ppm, except for the natalizumab Fd domain, where a 2 Da mass deviation was determined.

All domains were subjected to MD analysis by MALDI-ISD using sDHB matrix and an average sequence coverage (SC) of 89% was obtained excluding the natalizumab Fd (< 50%) and cetuximab (86.1%). In the case of cetuximab Fd, PNGase F deglycosylation brought the SC to 92.4%. Natalizumab showed an N-terminal match in one reference sequence until only c101. Software supported analysis allowed identifying another fully matched sequence, which was previously reported (1). It was differed by 3 point mutations with a mass shift of 2 Da. Comparison with the Wang sequence yielded 89.5 % SC in the MALDI-ISD spectrum.

These data combined sub-ppm mAb domain MW determinations with middle-down sequencing spectra of unprecedented quality. Most MD spectra provided an overlap of the N- and the C-terminal sequence readout with very few interruptions, such as proline –gaps.

Altogether the sub-ppm antibody domain MWs and the MALDI MD sequencing data provided a straightforward and very fast method to reliably validate bioparmaceutical sequences and to reliably detect and correct sequence errors.

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Hydrogen Deuterium Exchange LC-MS and Molecular Modeling of the TIMP-3 sulfated GAG (psHA) complex

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Keywords: TIMP-3, GAG, HDX, Hydrogen Deuterium Exchange, LC-MS

Einleitung

In a recent study we found that native and chemically modified Glycosaminoglycans (GAGs) strongly affect the activity of the ECM-degrading matrix Metalloproteinases (MMP) -1 and -2 which are known to be key regulators of tissue formation and remodeling of extracellular matrix and thus are interesting targets to facilitate wound healing processes. Furthermore we could show that the abundance of its regulators, the tissue inhibitors of Metalloproteinases (TIMP), is controlled by sulfated GAGs. However, whether there is a direct interaction of TIMPs and sulfated GAGs and whether this affects the binding to MMP is not known.

In this study the interaction of the persulfated hyaluronic acid (psHA) and TIMP-3 was studied by backbone amide H/D exchange mass spectrometry and molecular modelling.

Experimenteller Teil

TIMP3 with and without psHA was studied by hydrogen deuterium exchange. 20 pmol TIMP-3 was incubated with D2O for 1, 60 and 1440 minutes in the presence and absence of psHA. Experiments were performed on a Waters HDX Bench consisting of a Xevo G2S QTOF MS coupled to a Waters HDX Manager enabling ultra short LC gradients at 0°C and online pepsin digestion in a completely monitored and controlled environment. Sample preparation was consistent with previous studies[1]. A homology model of TIMP-3 was predicted using TIMP-3-ADAM-complex (N-Terminus) and TIMP-1 (C-terminus). This model was docked to psHA, using the Lamarckian-Genetic-Algorithm resulting in three representative pose models, and underwent molecular dynamics simulation with Free Energy calculations and PBSA electrostatic potential calculations.

Ergebnisse und Diskussion

We studied the H/D exchange of the backbone amides of TIMP3 in complex with and without psHA to determine the residues being affected by GAG binding. Using this approach we were able to obtain information about 87% of the TIMP3 sequence and achieved an average resolution of 4.5 amino acids. Conclusively, the amino acid sequences R20-A21, K22-Y39, T105-L106, S107-N113, Q155-H158, and I162-W171 were found to be most affected due to interaction with psHA. MD simulations for TIMP-3 clearly suggest a region with the positive electrostatic potential, which could be involved in GAG binding [2]. Among these residues K-76, K-165 and R-163 were included in the motifs suggested previously to be important for GAG-binding according to the mutagenesis study, which proposed two GAG binding regions on TIMP-3 at the termini [3]. Summarized we predict three distinct binding sites of TIMP-3 to be involved in GAG-binding. Binding site A comprises the amino acids R-48, G-49, F-50, R-84, K-123, L-125, R-163, K-165, binding site B contains R-20, K-22, K-42, K-45, K-76, Y-77, R-100, W-101, R-109, K-110, N-113 K-165 and R-173, while binding site C is formed by R-20, K-22, K-42, M-44, K-45, H-55, W-101, R-109, K-110 and N-113.Compared to molecular docking experiments sequences being affected due to binding overlap with 70% of binding sites B and C, whereas binding site A is fully covered with the exception of the undetectable peptides 117-133 which should contain two proposed amino acids involved in GAG-binding. In conclusion TIPM3 probably has three intermediate GAG-binding sites in total, however, none of these GAG-binding region do overlap with the MMP-binding region. Consequently, implants coated with sulfated GAGs potentially allow an enrichment of TIMP3 in the wound region to locally reduce the MMP activity and thus facilitate wound healing processes.

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Identification of C-termini in proteins and proteomes by charge-reversal derivatization and parallel use of multiple proteases

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Keywords: C-terminus; Derivatization; E. coli

Einleitung

Identification of protein C-termini in proteomes (C-terminomics) is still a challenging task. However, the knowledge of protein C-termini is important to elucidate proteolytic events or to identify C-terminal modifications. Strategies to study C-terminal posttranslational modifications (PTMs) are limited due to the low chemical reactivity of the carboxyl group. Additionally, its poor ionization efficiency often hampers identification of protein C-termini in bottom-up proteomics [1,2]. Based on a recently published strategy [3] we chemically derivatized carboxyl groups with ethanolamine (EA), N,N-dimethylethylenediamine (DMEDA) or (4-aminobutyl)guanidine (AG). After proteolysis C-terminal peptides were separated from internal ones via polymer based depletion.

Experimenteller Teil

Model proteins and an E. coli proteome were denatured, disulfides reduced/alkylated, and free amino groups were blocked by reductive dimethylation. Carboxyl groups of Asp, Glu and protein C-termini were protected via EDC/NHS-activation with EA, DMEDA or AG. Labeled proteins were proteolyzed with Trypsin and/or chymotrypsin in parallel and the neo-N-termini were blocked by reductive dimethylation. Internal peptides were depleted by condensation to polyallylamine (PAA) and the remaining peptides were analyzed by nano-LC-ESI-MS/MS using CID, HCD and/or ETD.

Ergebnisse und Diskussion

Derivatization at peptide level using digested model proteins showed a high labeling efficiency for all carboxyl groups of Asp, Glu and C-termini using EA and DMEDA, while the labeling with AG was slightly less efficient. At protein level the derivatization of C-terminal carboxyl groups was more efficient than for those of Asp und Glu residues. For peptides lacking basic amino acids we observed increased fragment ion coverage not only for b-ions but in particular for the corresponding y-ions. Depletion of internal peptides via PAA coupling allows significant reduction of the sample complexity leading to lower ion suppressing effects and therefore better ionization efficiencies for the remaining peptides. Applying this approach to an E. coli proteome - using EA- and DMEDA-derivatization in parallel - we identified 424 unique protein C-termini. Thereof, 267 are matching the genetically encoded canonical protein C-termini while the remaining 157 are potentially truncated forms originating from proteolytic processing in vivo.

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Identifizierung von intrazellulären Cisplatin-Protein Addukten mithilfe des fluoreszierenden Cisplatin-Analogon CFDA-Cisplatin

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Keywords: Cisplatin-Analogon CFDA-Cisplatin, Cisplatin, Cisplatin-Protein Addukte, Proteinmarkergitter, zweidimensionale Gelelektrophorese

Einleitung
Cisplatin ist ein breit eingesetzter Wirkstoff in der Krebstherapie. Derzeit erfolgt eine Behandlung mit Cisplatin bei Plattenepithelkarzinome an Kopf und Hals, Bronchial-, Darm-, Harnblasen-, Hoden- und Ovarialkarzinom. Seine Wirksamkeit ist aber durch toxische Nebenwirkungen sowie durch eine Resistenz von Tumorzellen gegenüber Cisplatin begrenzt.

Die zytotoxische Wirkung von Cisplatin beruht auf der Platinierung der DNA [1], aber nur ein geringer Anteil des zellulären Cisplatins gelangt zum Zielort [2]. Es wird angenommen, dass Cisplatin eine höhere Affinität zu schwefelhaltigen Aminosäuren wie Cystein und Methionin hat [3]. Im Inneren einer Tumorzelle gibt es für Cisplatin eine Reihe von möglichen Bindungspartnern. Daher kann für Cisplatin die Wechselwirkung mit Proteinen ein wichtiger Faktor für dessen intrazelluläre Verteilung, Elimination und Zytotoxizität sein [4].

Experimenteller Teil
Zur Identifizierung und Auftrennung solcher intrazellulären Cisplatin-Protein Addukte wurde mithilfe des fluoreszierenden Cisplatin-Analogon CFDA-Cisplatin [5] eine 2D-Gelelektrophorese etabliert. Dieses fluoreszierende Cisplatin-Analogon ermöglichte die Detektion bzw. Unterscheidung von intrazellulären Cisplatin-Protein Addukten.

Um die Komplexität und Auflösung von sauren, neutralen sowie basischen Proteinen zu erhöhen, erfolgte die Auftrennung mithilfe der 2D-Gelelektrophorese in teilweise überlappende immobilisierte pH-Gradienten (pH 4-7 und 6-10). Die detektierten CFDA-Cisplatin-Protein Addukte wurden mittels ESI-MS/MS identifiziert.

Ergebnisse und Diskussion
Mit dieser Strategie konnte der Elongation factor 1-alpha 1, die D-3-phosphoglycerate dehydrogenase und einige Protein-Disulfid-Isomerasen identifiziert werden.

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Posternummer: 25

Identify cellular mechanisms by accurately analysing the site-specific degree of phosphorylation of signalling proteins

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Keywords: Systems biology, phospho-proteomics

Einleitung

Mass spectrometry is the method of choice to determine the degree of protein phosphorylation in an accurate and site-specific manner. The activation status of individual signalling proteins or whole pathways can be characterized by determining the degree of phosphorylation at particular sites.

Here we present the principle strategy and a translational application of our innovative method for the robust determination of the degree of phosphorylation with maximal accuracy [1, 2]. The key advantage of our standardization method is the cheap and straight-forward generation of an isotopically labelled peptide/phosphopeptide mixture with a precisely defined molar ratio.

Experimenteller Teil

Such a mixture is spiked into the sample during the proteolytic digestion and helps to standardize the distinct physicochemical properties of the respective peptide and its corresponding phosphopeptide for the subsequent workflow steps such as clean-up, chromatography and ionisation. To determine the phosphorylation dynamics of the MEK/ERK module in response to hepatocyte growth factor (HGF) or interleukin-6 (IL-6) and unravel alterations in the network structure, we combined the application of our method for the accurate quantification of ERK1/2 phosphorylation at T and Y within the TEY-activation motif with mathematical modelling. We compared the dynamics of pathway activation in primary mouse hepatocytes with the dynamics in the immortalized human keratinocyte cell line HaCaT-A5.

Ergebnisse und Diskussion

Quantitative analysis and mathematical modelling resulted in the prediction that the prevalence of mono-threonine phosphorylated ERK rather than the doubly phosphorylated ERK is indicative for dysregulated proliferation. Finally, the differential analysis of tumor-free and tumor-containing human hepatocellular carcinoma samples confirmed this conclusion [3]

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Posternummer: 26

Improved selectivity for the quantitation of proteins in complex biological samples by the use of the SelexIONTM technology

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Keywords: SelexION, Peptide, Quantitation

Einleitung

The knowledge about changes in protein expression is critical to understand biological systems and multiple reaction monitoring (MRM) is widely used for the quantitation of proteins. Even though MRM is a highly selective scan mode, complexity of biological samples could cause issues due to increased background or false positive signals. SelexIONTM technology is a planar differential mobility device, which can be attached to the front end of a QTRAP® system. Separation of peptides by differential mobility spectrometry (DMS) increases the selectivity for MRM quantitation. In this study we examined and optimized the use of SelexIONTM in combination with nano-LC MRM.

Experimenteller Teil

A 6500 QTRAP® mass spectrometer was equipped with SelexIONTM and a Nano III ion source. Manual nano-spray infusion of a Beta-Galactosidase digest was performed to test the influence of source parameters as well as DMS parameters on the intensity and separation resolution using SelexIONTM. Nano-LC MRM was done to get the final DMS MRM method with optimized parameters. Finally Beta-Galactosidase, spiked in HeLa cell background, was analyzed either with or without DMS by nano-LC MRM to examine the increase in selectivity.

Ergebnisse und Diskussion

The source parameters using SelexIONTM were found to be similar to normal nano-spray, except the nebulizer gas pressure, which should be lower to get best sensitivity as well as peak resolution. The temperature in the DMS cell had the highest influence on the peptide separation using DMS without having a negative influence on the intensity. The comparison of Beta-Galactosidase peptide detection in HeLa cell background using nano-LC MRM with and without SelexIONTM clearly showed a much lower number of background peptides using DMS. The overall MRM background noise was also much lower, which led to a better S/N in peak detection.

Referenzen

Increasing peptide signal intensity in MALDI MS by PPG as matrix additive.

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Keywords: MALDI, signal intensity, Polypropylene glycol

Einleitung

There are numerous approaches to improve MALDI MS results especially in view of signal intensity. One strategy in proteomics experiments is to coat the target with graphite [1], add an additive [e.g. 2] or the sample tubes [3] with PPG to avoid sample loss on surfaces. We use PPG as matrix additive to enhance the signal intensity of peptides using MALDI-MS.

Experimenteller Teil

A matrix solution containing 3.0 mg/mL α-cyano-4-hydroxycinnamic acid in 70:30 ACN/0.1 % TFA was mixed with a clear aqueous solution containing 1 mg/mL polypropylene glycol (PPG 4000; Sigma). A matrix solution without PPG served as a standard. A serial dilution with matrix solution starting from a total PPG concentration of 10 ng/mL to 0.1 fg/mL was prepared to investigate the effect of PPG on the signal intensity. The experiments were performed with a mix of four different peptides (Bradykinin, Glu-1-fibrinopeptide B, Neurotensin, Substance P; c=100 pg/µL).

Ergebnisse und Diskussion

The peptides and the matrix solutions were mixed 1:1 and each mixture was spotted 10 times. Spectra (100 scans) were obtained on a MALDI Orbitrap –XL. The averaged absolute intensities (peak height) of the peptides in each matrix mixture were divided by the standard intensities.

The polypropylene glycol concentration significantly affected the measured peptide signal intensities. The PPG concentration was reduced until no further effect was observed. With the highest PPG concentration of 10ng/ml (7.5 pg PPG per spot) signal intensities of all peptides were reduced by more than 50% compared to the standard matrix solution. By further dilution of the additive the signal intensity returned to standard level and exceeded it by more than 50% at a PPG concentration of 0.1 fg/ml. Bradykinin was only marginally affected by PPG, whereas Neurotensin and Substance P experienced the greatest benefit.

In high PPG concentration, the crystallisation is disturbed. The increasing peptide signal intensities at low PPG concentrations might be explained by the longer time the spots need to dry compared to those without PPG. It is suggested that this is beneficial for an improved incorporation into the α-cyano-4-hydroxycinnamic acid crystals. A combination with other matrices may also be applicable for higher signal intensities and need to be further investigated.

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Isobaric Mass Tagging Quantitation using Q Exactive instruments – Approach and Expectation

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Keywords: Protein quantitation, isobaric mass tagging, Orbitrap

Einleitung

Isobaric mass tagging has become a common technique in mass spectrometry for relative quantification of proteins. However, there are a couple of factors that impede reliable quantification of complex samples. One of these factors is co-isolation of peptides, which leads to systematic under/overestimation of quantitative ratios of the isobaric tags [2]. Co-isolation interference has been addressed using various kinds of approaches, such as using MS3 analysis [3]. Quadrupol Orbitrap benchtop instruments can be used to address some of these challenges. The effect of several parameters on the protein quantification accuracy and precision is discussed.

Experimenteller Teil

Digested HeLa or E.coli cell lysates were labeled with TMT10plex™ reagents according to manufacturer's instructions. Aliquots from all ten channels were mixed in different ratios and separated using the Thermo Scientific™ EASY-nLC 1000™ HPLC system and 50 cm EASY-Spray™ PepMap™ RSLC C18 column. Gradient lengths were from 2 to 4 hours using a flow rate of 250-300 nl/min. Labeled samples were analyzed on the Q ExactiveTM HF and Q ExactiveTM Plus mass spectrometers. Raw data files were processed using Thermo Scientific Proteome Discoverer™ version 2.0 software. The peptides/proteins were identified using the Sequest HT® search engine. Parameters such as MS2 injection time, AGC target, normalized collision energy (NCE) and gradient length were adjusted to understand their effect on the quantitation.

Ergebnisse und Diskussion

The influence of several parameters on the number of quantified proteins and precision of quantitation was investigated. For example, narrowing the isolation width results in decreased interference with only minimal loss of the total number of identified proteins due to very efficient quadrupole isolation and ion transmission. Increasing the MS2 injection time leads to higher number of protein quantified, as well as better quantitation accuracy and precision. In addition, increasing MS2 AGC target and NCE can both help on reporter ion statistics for better quantitation. Increasing LC gradient length further improves precision and reproducibility of quantitation but requires either injecting double amount of sample or longer injection times. After optimization, the best settings were used to quantify proteins from 2ug equimolar TMT10plex HeLa digest. From duplicate experiments of 2 hours gradient about 4000 proteins were identified with FDR of1 % and quantified with average ratios CV below 17%.

To assess the improvements on precision of quantitation, TMT3plex™-labeled HeLa samples were equally mixed (1:1:1) and spiked with E.coli labeled with TMT6plex™ reagents mixed at different ratios (20:10: 1:1:10:20). The sample (1 ug of E.coli + 500 ng HeLa digest) was then analyzed using optimized methods. Median ratio of up to 10 was observed for 1:20 expected ratio in presence of HeLa interference.

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Large Scale Quantitation of Stable Isotope Labelled Proteomes Using Retention and Drift Time Profiling

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Keywords: Proteomics, Quantitation, IMS, DDA/DIA, SILAC

Einleitung

The use of stable isotope labelling is routinely applied in LC-MS proteomics based quantitation. Labels are incorporated metabolically, enzymatically, chemically or by stable isotope labelling. The comparison of multiple samples necessitates a higher degree of label multiplexing, which in turn requires higher separation power LC systems, more sensitive, faster and advanced MS to acquire more and better quality product ion spectra, and informatics tools that can take advantage of the high resolution and mobility separation afforded by modern MS. Here, the principle and application of novel informatics is described to enable stable isotope labelling quantitation using commercial software originally developed for large scale quantitation of label-free LC-MS data, incorporating novel retention and ion mobility drift time profiling based scoring algorithms.

Experimenteller Teil

High resolution precursor and product ion LC-MS data were acquired in DDA, DIA or IM-DIA mode. Data were peak detected with Progenesis QI using default noise filtering and a zero intensity threshold value. A search approach was utilized whereby multiple fixed modification searches were sequentially conducted to minimize FDR and mapped to feature data. Isotopic profiles appended with identifications were exported to ProteoLabels. Modification masses were determined automatically by ProteoLabels and m/z, retention and drift time tolerances set based on the observed distributions. Next, peptide pairs were detected, allowing for prior identification of only one of light or heavy, and pair scores calculated based on mass shift, chromatographic and drift profile. Protein grouping was conducted and weighted average ratios reported.

Ergebnisse und Diskussion

Six data sets, five SILAC and one dimethyl, were used to evaluate Progenesis QI/ProteoLabels quantitative analysis of isotopically labeled LC-MS data on accuracy, precision and coverage. Ionic peptide drift time information was available for the dimethyl labeled data and four of the SILAC data sets. A number of sample sets were created with expected ratios for validating the quantitative results. The informatic analysis of technical LC-MS replicates was conducted to determine reproducibility. Progenesis QI affords co-detection across samples and data sets, including both technical and biological replicates, as well as time-course samples, which increased on average the number of detected isotopic clusters by 2.1 fold compared to analysis of data on a single run basis. In addition, the number of quantifiable isotopically labeled peptide pairs by ProteoLabels was found to be significantly increased by a factor of 1.8, requiring a minimum of two peptides for quantitation, when compared to commercially available software for the analysis of DDA and DIA data, including IM, with subsequent protein amino acid coverage increase. Median weighted average protein and peptide pair ratio values determined by Progenesis QI/ProteoLabels analysis were found to be in good agreement with previously reported results for the data sets investigated. Combined retention and drift time similarity profiling improved the peptide pair scoring, thereby reducing quantitation FDR and improving quantitation precision.

Referenzen

Metal tagging of antibodies

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Keywords: ICPMS, MeCAT, Click chemistry, Antibody

Einleitung

Not only qualitative but also quantitative determination of biomolecules is a topic of big importance, since it reflects status and changes in biological system. [1] As far as quantification is concerned, elemental mass spectrometry, namely inductively coupled plasma mass spectrometry (ICPMS) is known to be a perfect tool for this purpose, it is also known for its high sensitivity, linearity and matrix robustness. Numerous labelling approaches for antibody detection have been developed so far. However, development of new tags is still of great importance. In the given work, we present an approach for Metal-Coded Affinity Tags (MeCAT) labeling of antibodies through pre-click reaction procedure with further sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation and ICPMS quantification.

Experimenteller Teil

In this study, a strategy for antibody labelling with further SDS-PAGE separation and ICPMS quantification is utilized. Firstly, MeCAT is functionalized with trans-cyclooctene (TCO) derivative through NHS chemistry reaction. Secondly, antibody is labelled with bis-sulphone reagent functionalized with tetrazine group, followed by the attachment of metal label to antibody through tetrazine ligation. The bis-sulphone reagent can restore a covalent linkage between the two cycteins, unlike maleimide based conjugates. It was also found out that the selective reduction of disulfide bridges is of crucial importance to perform re-bridging of antibody afterwards.

Ergebnisse und Diskussion

A novel approach for metal labelling of antibody has been developed. It is based on a strategy including selective reduction of disulfide bridges, labelling with bridging bis-sulphone reagent followed by conjugation via Inverse electron demand Diels–Alder reaction. The functionalization of MeCAT with TCO was optimized to enhance the yield of coupling product and suppress the hydrolysis of N-Hydroxysuccinimide which results in undesired side products. Another important issue is the choice of the appropriate reducing agent. In terms of this work two reducing agents, namely tris(2-carboxyethyl)phosphine (TCEP) and 2-mercaptoethanolamine (2-MEA) were compared. It has been found that utilization of 2-MEA as reducing agent provides the selective reduction of disulfide bridges, unlike in case of usage of TCEP the reduction is not selective. Quadrupole time of flight mass spectrometry (Q-TOFMS) was applied for determination the degree of labelling. The crucial difference from reported MeCAT labelling approaches of antibodies is that bis-sulpone reagent can restore a covalent linkage between the two cycteins, unlike maleimide based conjugates.

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Multi-Omics Analysis of Primary Cytotrophoblasts from Second Trimester and Term Placentas

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Keywords: SWATH, cytotrophoblasts, multiomics

Einleitung

During human pregnancy, a subset of placental cytotrophoblasts (CTBs) differentiates into cells that aggressively invade the uterus and its vasculature, anchoring the progeny and rerouting maternal blood to the placenta. Defects in this process are the hallmark of the pregnancy complication preeclampsia. While disease-associated genes or transcripts may serve as useful biomarkers, they are not necessarily predictive of disease mechanisms.

Tools exist in each of the omics fields to profile changes. However there is an urgent need to build tools and infrastructure that will allow integration of these data streams. In this Pilot study we performed global proteomic and transcriptional profiling of CTBs to gain further understanding of their differentiation during pregnancy using a seamless processing for the entire project.

Experimenteller Teil

Primary CTBs were isolated using collagenase and trypsin digestion and Percoll gradient centrifugation. For transcriptomics, a second set of CTBs were analyzed using RNAseq. For proteomics analysis, cells were lysed and digested with trypsin. A portion of each sample was pooled and subjected to 2D fractionation for generation of an in-depth ion library.

SWATH and information dependent (IDA) acquisition was performed on a TripleTOF® 6600 system coupled to a NanoLC™ 425 System.

IDA fractions were processed using ProteinPilot™ Software 5.0 to generate an ion library. The SWATH MS data were processed using OneOmics™ applications (SCIEX) in BaseSpace (Illumina). iPathwayGuide (Advaita) was used to compare protein and RNA levels for pathway and gene ontology analyses.

Ergebnisse und Diskussion

Using a 2D generated ion library, ~3000 proteins were quantified from the SWATH acquisition experiment. From these, ~400 proteins were differentially expressed between the 2nd Trimester CTB cells vs the full term CTB cells using a fold change confidence of 75% or greater.

These results from the OneOmics™ applications was then seamlessly imported in iPathwayGuide for RNASeq expression data comparison to the two proteomics 2ndTrimester vs Term contrasts. 9GO terms were found to be significant in all three contrasts. A few of these GO terms make sense for the biology, giving us confidence in the strategy for the Pilot study, such as Extracellular Matrix Disassembly, Angiogenesis, Response to Hypoxia.

By selecting a significant ontology, the measured genes/proteins can be easily visualized. In some cases, the expression changes are concordant between the RNASeq and SWATH acquisition data, like with HSPG2 (perlecan) protein showing more expression in the 2nd Trimester samples vs full term samples. This protein is known to be reduced in pregnancy complications like preeclampsia. It is thought to be involved in angiogenic or inflammatory processes as well within the placenta.

In other cases, we see different expression behaviors between RNA and protein as illustrated with the Nidogen (entactin – NID1) protein where we see down-regulation of protein in the 2nd trimester but minimal change in the RNA. This is a basement membrane glycoprotein that promotes trophoblast adhesion. Fibrillin 1 (FBN1) is also discordant with RNA showing no change while the protein levels were lower in the 2nd trimester samples.

Referenzen

Overcoming the challenges in Data Independent Acquisition (DIA) via high resolution accurate mass Orbitrap based mass spectrometer

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Keywords: Data Independent Acquisiton, Orbitrap, Urinary proteome

Einleitung

The promises of data independent acquisition (DIA) strategies are a comprehensive and reproducible data collection for large-scale quantitative proteomics experiments. However, the wide isolation window (usually >10Da) of DIA experiments co-isolates and co-fragments multiple peptides, resulting in highly complex DIA MS/MS spectra and makes the DIA data analysis challenging. To accurately identify and precisely quantify thousands of proteins per DIA experiment, the completeness and specificity of spectral library, the mass accuracy of the data, and the technical variance in quantitation play important roles. In this work, we utilize Thermo ScientificTM Q Exactive HF™ mass spectrometer for DIA LC-MS/MS experiments to study the urinary proteome, and demonstrate how high resolution/ accurate mass spectrometry is employed to overcome these DIA challenges.

Experimenteller Teil

Urinary sample were desalted, trypsinized and were measured multiple times with both data-dependent acquisition (DDA) and DIA experiments on a nanoLC online coupled to Q Exactive series system. The DDA data files were searched against MaxQuant and Sequest HT search engine. The results were filtered by 1% FDR and used to build up spectral library. DIA data were analyzed against this sample specified spectral library as well as the public available human spectral library (1) via the Spectronaut (Biognosys) software.

Ergebnisse und Diskussion

To elucidate the effect of the spectral library on data analysis, we prepared the libraries that were generated from DDA data of this urine samples and compared the results to the same DIA samples analyzed with a publicly available human spectral library (1). This library covers the majority of the human proteome, including the vast majority of the identified urinary proteins. The urine sample-specified spectral libraries are built based on the multiple DDA runs against the MaxQuant and Sequest HT database, which results in > 2000 proteins and ~ 20,000 peptides (1% FDR). The results show a higher reproducibility of peptide/protein identification and quantification when using the urinary spectral library. This finding underscores the importance of using a sample-specific spectral library.

The application of wide isolation windows (>10 Th) in typical DIA experiments results in complex MS/MS spectra. During data analysis, the ion chromatograms of multiple fragment ions are extracted and aligned for peptide detection and quantification. To separate the analyte of interest from interferences, a highly accurate mass of the ions is crucial. We applied different mass tolerances for the data analysis (50ppm, 20ppm, 10ppm, and 5ppm) and the results show that 10 ppm mass accuracy is minimum requirement for an accurate detection of peptides.

A high technical reproducibility is essential for a precise quantification of biological compounds. We assessed the technical reproducibility of the DIA method by analysis of the same sample multiple times and calculated the CV of peptide and protein quantification using the sample-specific spectral library. The median CV was below 10% and more than 85% of the peptides and more than 80% of the proteins were quantified with a CV better than 20%, indicating an excellent technical reproducibility.

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Posternummer: 33

Proline Hydroxylation in an HEK and CHO produced IgG based-fusion protein

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Keywords: Proline Hydroxylation, Fusion protein, LC-MS/MS, Orbitrap Fusion, PTM, maXis

Einleitung
Protein hydroxylation is a post-translational modification (PTM) that can occur at the sites of proline and lysine involving the conversion of a C-H group into a C-OH group (ΔMass: +15.99 Da). Hydroxylation of proline involves the enzyme prolyl hydroxylase which catalyze the reaction in the lumen of the endoplasmic reticulum of eukaryotes. Although rarely reported for therapeutic proteins, hydroxyproline ((2S,4R)-4-Hydroxyproline, L-hydroxyproline, HyP) is not rare to nature as it comprises about 4% of all amino acids found in animal tissue e.g. being a key factor in stabilizing collagens. Proline hydroxylation requires Vitamin C (ascorbic acid) as cofactor.

Experimenteller Teil
The hydroxy groups of hydroxyproline form hydrogen bonds with other amino acids to tightly bind the collagen proteins to each other. The PTM of the fusion protein was discovered by off-line ESI-QTOF-MS using a Bruker maXis G4 QTOF, and quantified by XIC evaluation following LC-MS/MS peptide mapping performed on a Orbitrap Fusion (Thermo Fisher Scientific).

Ergebnisse und Diskussion
Here we report high levels (21-45%) of site-specific hydroxyprolines in an IgG based-fusion protein produced in CHO and HEK. The influence of the presence of the hydroxyproline was analyzed by surface plasmon resonance.

Referenzen

Posternummer: 34

Quantitative proteome analysis of host-pathogen interactions in Caenorhabditis elegans

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Keywords: High pH reversed-phase chromatography, 2D-LC-MS, Innate immune system, host-pathogen interaction

Einleitung

Understanding the molecular mechanisms involved in the interaction of higher organisms with their microbiota is an important key for understanding host-parasite coevolution and for the identification of processes related to both the maintenance of homeostasis and the development of diseases. Caenorhabditis elegans can be infected by a plethora of pathogens and the nematode has emerged as a powerful surrogate host to model microbial infectious diseases in a non-vertebrate, for the study of innate immunity and host-pathogen interactions [1]. We analyzed the protein abundance profile of the nematode upon microbial challenge with the pathogenic bacterium Bacillus thuringiensis (Bt) in comparison to a non-pathogenic Bt strain.

Experimenteller Teil

C. elegans was grown on agarose petri dishes seeded with E. coli cells either as a monoculture or supplemented with spores of a pathogenic Bt strain or a non-pathogenic Bt strain, respectively in four independent replicates. A novel method for the preparation of defined developmental stage C. elegans samples was developed by nylon filter assisted harvesting. Relative protein quantification was performed using isobaric labeling combined with 2D-LC-MS analysis. Tryptic peptides of extracted proteins were iTRAQ-labeled and separated over a powerful high resolution 2D-LC separation scheme using fraction concatenation.

Ergebnisse und Diskussion

More than 3,600 proteins were quantified, 288 of which showed altered abundances, implicating protein classes such as lectins, lysozymes, and transthyretin-like proteins to be involved in the nematodes innate immune defense. A number of gene products previously only associated with the immune defense by transcriptomic profiling could be verified at the protein level. Moreover, several other protein classes such as proteases, proteins related to autophagy and apoptosis, structural proteins, and proteins involved in chromatin organization were differentially abundant.

The results provide an overview of the physiological response towards a pathogen at protein level in the important model organism C. elegans, giving insights into the complexity of host-pathogen interactions.

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Rapid Detection of Deamidation in Monoclonal Antibodies using Ultrahigh-Resolution QTOF Mass Spectrometry

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Keywords: antibody, subunit analysis, deamidation profiling, UHR-QTOF

Einleitung
Characterizing the heterogeneity in therapeutic proteins is a fundamental task of all product development and commercialization phases, and in discovery research for molecular construct selection. Product heterogeneity is caused by posttranslational modifications (PTMs) like glycosylation and N-/C-terminal truncations/extensions, and by chemical degradation by production process or storage conditions, such as oxidation and deamidation. Asn deamidation is particularly challenging to detect on intact proteins given the +0.984 Da mass addition, and currently is characterized with bottom-up approaches. As proteolytic digests take considerable time and can introduce additional method-related deamidation, we developed a rapid method that utilizes middle-up antibody fragment isotopic distributions.

Experimenteller Teil
A model system for deamidation of biologics was generated: The antibody adalimumab (Abbvie) was partially reduced with DTT resulting in the cleavage of the interchain disulfide bonds only. The sample was then split into two aliquots which were differently alkylated, one with iodoacetamide the other with iodoacetic acid. As a result, the masses of the light chains (LCs) of the two aliquots differ by 1 Da. The two aliquots were mixed in different ratios and analyzed by LC-QTOF providing isotopic resolution and accurate monoisotopic mass determination of LCs and heavy chains.

Ergebnisse und Diskussion
Highly accurate mass measurements are required to reliably identify PTMs with a small mass shift like deamidation on protein subunits like LCs and HCs of monoclonal antibodies. QTOFs of the latest generation achieve a resolution which allows for isotopic resolution of HCs in the mass range of 50 kDa in routine HPLC-MS workflows and the subsequent determination of their monoisotopic masses with accuracies better than 3 ppm. For the three antibody subunits which are generated by the IdeS enzyme with masses between 23 and 28 kDa, mass accuracies of 1 ppm and better are typically achieved.

MS detection of deamidation at the antibody subunit level was shown using the model system of the pure aliquots. The average mass deviation between the measured and expected monoisotopic masses derived from the amino acid sequence was -0.48 ppm. Using a 30 min gradient, the differently alkylated LCs show a retention time difference of 12 s. This retention time difference was not resolved in mixtures with a mixing ratio of 1:3, 1:1 and 3:1, however was reflected in a broadening of the chromatographic peak. The monoisotopic masses derived from the averaged mass spectra of the broadened chromatographic peak reliably reflect the mass of the dominant species with the same accuracy as for the pure compounds. However, due to the different retention times of the species, the occurrence of the lower abundant species can be elucidated by analyzing each spectrum of the chromatographic peak separately.

Referenzen

Rapid method for monitoring monoclonal antibody (mAb) production in Biotechnological processes using quantitative MALDI-TOF-MS

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Organisation(en): ETH Zurich, Schweiz

Keywords: MALDI-MS, Antikörper

Einleitung
MAb process monitoring using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) provides new insights on process stability, efficacy and viability when looking at intra and extracellular protein levels. MAbs and their production processes have undergone an impressive development from immunology concepts formulated over a century ago by Paul Ehrlich, to their nowadays-widespread clinical applications. Current methods for monitoring mAb production are based on separation techniques like size-exclusion chromatography (SEC), and liquid chromatography (LC), and are often time consuming and expensive.

Experimenteller Teil
Here we demonstrate a fast relative quantification MALDI-MS monitoring method for intact mAbs with a mass accuracy of < 0.5 % at 150.000 Da based on internal calibration steps. MAb samples are aliqouted without prior sample preparation together with a UV-absorbing matrix and an internal standard protein onto a microarray for mass spectrometry (MAMS) chip. Subsequent analysis (number of replicates n = 10) is performed on a commercial MALDI TOF instrument equipped with a high mass conversion dynode detector, in less than 1 minute.

Ergebnisse und Diskussion
The intact mAb and corresponding building blocks are identified and quantified in a relative fashion, which for the first time allows monitoring minor changes and inconsistencies in the overall production process. The linearity of the method is shown in a dilution experiment with a purified universal mAb. Fed batch and perfusion reactor cell cultures are investigated to prove the applicability of the method regarding, robustness and high throughput capabilities. The results of the monitoring experiment clearly demonstrate the expected trends and have been confirmed and cross-validated by HPLC-UV measurements. The developed method is superior to existing methods regarding time and costs.

Referenzen

Resistance mechanisms against antimicrobial peptide apidaecin 1b identified in E. coli using bottom-up proteomics

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Keywords: Antimicrobial peptide, Resistance, Bottom-up proteomics

Einleitung

Proline-rich antimicrobial peptides (PrAMPs) are promising lead structures to treat (multi-resistant) human pathogens1,2. In face of their high in vivo efficacies in different animal models, we studied here the proteomes of Escherichia coli strains with induced apidaecin 1b resistances to obtain further insights into modes of action and potential mechanisms of resistances. Therefore, the proteomes of the different resistant E. coli were quantified relative to the wild-type strain.

Experimenteller Teil

E. coli BL21AI was incubated in vitro with increasing concentrations of apidaecin 1b over ten passages to isolate less susceptible strains. The proteomes of the wild-type and resistant bacteria were examined after trypsin digestion of soluble and membrane protein fractions using a bottom-up proteomics on an ESI-LTQ-Orbitrap XLTM mass spectrometer coupled on-line to nanoRP-UPLC. Peptides identified in either cell lysate were relatively quantified using ProgenesisQI software and subsequently used to compare the protein expression levels between both E. coli strains

Ergebnisse und Diskussion

The MIC values of apidaecin 1b against E. coli BL21AI increased from initially 1 µg/mL to finally 256 µg/mL, which was considered to be a stable resistance, as the MIC values remained stable afterwards in the absence of PrAMPs. A quantitative comparison of the resistant and non-resistant strains indicated 48 proteins in the soluble fraction ("cytosolic proteins") and 13 proteins in the membrane fraction at significant different quantities. Knock-out mutants of four genes encoding the proteins with the largest decrease in expression levels were significantly less susceptible to apidaecin 1b that the corresponding wild-type strain BW25113, confirming their substantial role in the underlying resistance mechanism. Especially interesting appeared the type-I fimbriae (or adhesins) that are important for colonization of various host tissues and are thus considered as bacterial virulence factors.3 These virulence factors were virtually absent in the resistant strain, which showed consequently a significantly lower tendency to form biofilms in vitro than the wild-type strain. The reduced susceptibility could be explained by proposing that apidaecin 1b binds to the mannose-specific fimbrial FimH adhesion. The high peptide concentration on the bacterial surface should favor its cellular uptake, which might be deteriorated in non-fimbriated species leading to reduced uptake and thus lower activities. Importantly, the resistance mechanism identified here should reduce the virulence of E. coli considerably or even lead to non-virulent strains, which would therefore not challenge therapeutic treatments.

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Straightforward differentiation between isobaric peptides during disulfide bond characterization

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Organisation(en): Roche Diagnostics GmbH, Deutschland

Keywords: disulfide bond characterization

Einleitung

The correct formation of disulfide bonds is crucial for the biological function of a protein. Thus, the mapping of disulfide bonds and the determination of unpaired, mispaired or modified cysteines are of major importance during the characterization of therapeutic proteins.

Here we report our experiences on disulfide bond characterization using peptide mapping and the DBond software.

The characterization method was set up to detect the expected, correctly linked dipeptides as well as incorrectly paired dipeptides, free thiols and cysteines modified by small thiol containing molecules like cysteine, homocysteine and glutathione. These small thiol containing molecules may originate from the fermentation broth or from the glutathione (GSS/GSH) redox system present during the renaturation process.

Experimenteller Teil

The characterization method was set up to detect the expected, correctly linked dipeptides as well as incorrectly paired dipeptides, free thiols and cysteines modified by small thiol containing molecules like cysteine, homocysteine and glutathione. These small thiol containing molecules may originate from the fermentation broth or from the glutathione (GSS/GSH) redox system present during the renaturation process.

Here we show our observations regarding the differentiation of two isobaric species using their collision induced dissociation (CID) fragmentation patterns. By analysis of the fragmentation spectra using the DBond software we derived a fragmentation behaviour selective for glutathionylated peptides.

Ergebnisse und Diskussion

Here we describe the differentiation of two isobaric disulfide linked species - peptide linked to GEC* versus glutathionylated γEC*G linked peptide - using their collision induced dissociation (CID) fragmentation patterns. By analysis of the fragmentation spectra using the DBond software we derived a fragmentation behaviour selective for glutathionylated peptides.

DBond was ideally suited for this differentiation because it allows the identification of fragments of both of the two disulfide bonded species whereas other methods only consider monopeptide fragments and C-S or S-S bond cleavages [6] but don‘t account for Glutathione being a tripeptide which can also undergo fragmentation. The base signal fragment from a doubly charged precursor of a γEC*G bonded dipeptide is selective because it includes the y2-fragment. It can be used as a marker ion and therefore allows the direct distinction from GEC bonded peptides. The fragment pattern of triply charged precursors also contains this selective ion but in these cases is not the base signal.

Knowledge of this fragmentation behaviour can serve to differentiate between (in-)correctly paired peptides and glutathionalyted peptides in a straightforward manner. So far we have confirmed the selective fragmentation characteristics described here in various samples of disulfide linked proteins.

Therefore, we propose the y2-fragment of γEC*G to be used as an universal marker ion for glutathionylation in CID spectra. This marker ion is of special interest for the disulfide bond characterization of therapeutic monoclonal antibodies where a mix-up between the C-terminal LC peptide GEC and glutathionylation can occur.

Referenzen

Structural Analysis of Protein-Protein Interactions in the Polyadenylation-Complex by Chemical Crosslinking and Mass Spectrometry

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Organisation(en): MLU Halle, Deutschland

Keywords: Proteincomplexes, Cross-links, High-resolution-MS

Einleitung
Messenger RNA precursors (pre-mRNAs) undergo different processing steps, including splicing, 5'-end capping and 3'-end cleavage, and polyadenylation [1]. Pre-mRNAs get cleaved downstream of the poly(A) signal (often AAUAAA) and polyadenylated (up to 250 adenosin residues) by a multi-protein complex, which consists of different subcomplexes such as the cleavage stimulation factor (CstF), the cleavage and polyadenylation specifity factor (CPSF), cleavage factor I and II (CF Im; CF IIm) and several others. The complete compositon, structure and mechanism of the polyadenylation machinery is still unclear. To adress this issue, the subcomplexes are analysed by chemical cross-linking in combination with high resolution with mass spectrometry [2].

Experimenteller Teil
The (different) protein complexes, involved in 3'-end processing of pre-mRNAs, were recombinantly expressed in baculovirus-infected insect cells and purified by affinity and anion exchange chromatography. Chemical cross-linking of purified protein complexes was carried out with the homobifunctional amine-reactive and MS/MS-cleavable “urea-linker” [3].The cross-linked complexes were in-gel digested and analysed by nano-HPLC/nano-ESI-Orbitrap mass spectrometry (UltiMate 3000 RSLCnano/Orbitrap Fusion Tribrid, Thermo Fischer Scientific). Fragmentation of the urea-linker (by HCD or ETHcD combined fragmentation methods) generated specific fragment ion patterns for each cross-linked peptide (Δ 26u) that were analyzed by the customized software tool MeroX [4].

Ergebnisse und Diskussion
Results

Proteins were expressed and purified to homogenity and the complexes show expected RNA binding affinity in filter-binding assays.

SDS-PAGE analysis of cross-linked ternary CstF complex shows a single high molecular weight protein-complex. In-gel digestion and MS analysis of the cross-linked sample resulted in the identification of eight intramolecular cross-linking products within CstF-77, two intermolecular cross-linking products from CstF-64 to CstF-77 and one intermolecular cross-linking products from CstF-50 to CstF-77.

The CF Im complex consists of two subunits that assemble into a tetramer. For chemical cross-linking the urea linker was used again. In the SDS-PAGE analysis homodimers of the small subunit als well as tetrameric cross-linking products were identified.

Outlook

Further cross-linking experiments will be done to gain insights into the mammalian 3'-end processing complex.The identified cross-linking sites could be used as molecular rulers to generate more precise models of these subcomplexes. These studies will help to create a interaction map of the complexes and the identify the interaction sites of the proteins. Based on these, site-directed mutagenesis experiments could be performed to validate the results.

The ultimate objective is to elucidate the underlying mechanismsof the polyadenylation.

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Structural characterisation of new peptide variants produced by cyanobacteria using positive ESI-MSMS

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Keywords: peptides from cyanobacteria ESI-MSMS

Einleitung
Novel bioactive peptides from cyanobacteria are attracting increasing attention in the search for new therapeutics. Microviridins are cyanobacterial depsipeptides composed of proteinogenic L-amino acids that contain intramolecular ω- ester and ω- amide bonds. Microviridins belong to the class of ribosomally synthesized and posttranslational modified peptides (RiPPs) and are potent inhibitors of serin- proteases.[1] Correctly cyclised and processed microviridin B could be heterologously expressed from a fosmid library in Escherichia coli.[2]

In this study liquid chromatography electrospray ionisation coupled to high resolution quadrupole time of flight tandem mass spectrometry was applied to investigate a novel group of cyanobacterial RiPPs

Experimenteller Teil
For the expression of promising novel peptides an inducible minimal expression platform encoding the genes for the putative precursor peptide and the modifying enzymes was generated. After heterologous expression in E. coli the resulting peptides were separated from the modifying enzymes via Amicon centrifugal filters . Liquid chromatography –electrospray ionization tandem MS analysis in combination with multiple complementary enzymatic digestions (trypsin, pepsin and chymotrypsin) were used to verify amino acid sequences and identify the posttranslational modifications. On the basis of a series of tandem –MSMS and MRM experiments the structures of the new compounds were deduced. Multiple collision induced dissociation experiments were performed by varying the collision energies to obtain fragment ions of interest at high abundance.

Ergebnisse und Diskussion
Genome mining of selected cyanobacterial strains has unraveled the presence of miroviridin-related gene clusters encoding enzymes of the ATP-grasp ligase family in direct neighborhood of putative RiPP precursor genes. Based on these bioinformatic studies we expect the formation of intramolecular ester- or amide bonds within the novel peptides. These bonds are formed via lactam, and lacton linkages between ω- carboxy groups of aspartate and glutamate residues and ε- amino- group of lysine and the hydroxyl groups of serine and threonine residues.We found that the double and triple protonated linear peptides can be easily sequenced by tandem mass spectrometry. In contrast the tandem mass spectra of the cyclic peptides are very difficult to interpret. Cyclic peptides are known for initial cleavage of the amide bonds, leading to the formation of all possible b-ion linear sequences having a free N-terminus by forming C-terminal b- oxazolon ions.[3] In case of the modified peptides the MSMS spectra were interpretable in terms of primary ring opening and subsequent fragmentations of linear peptide ions giving rise to series of immonium like b-ions via loss of CO.[3] The linear bX-type ions then dissociate into a series of fragment ions providing unambiguous sequence information. To date we propose a ω-lacton ring between the carboxy group of aspartate and the hydroxyl group of serine. Further modifications may be present and subject of ongoing investigations.

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Posternummer: 41

Structural insights into retinal guanylylcyclase-GCAP-2 interaction determined by cross-linking and mass spectrometry

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Keywords: chemical cross-linking, protein interaction

Einleitung

Retinal guanylylcyclases (ROS-GC 1 and 2) are regulated via the intracellular site by guanylylcyclase-activating proteins (GCAPs) dependent on intracellular calcium concentrations. However, the exact mechanisms of how GCAPs activate their target proteins is still not fully characterized as only structures of non-activating calcium-bound GCAP-1 and -2 are available. In this work, we apply chemical cross-linking in combination with mass spectrometry to study the interaction between calcium-bound and calcium-free N-terminally myristoylated GCAP-2 and the catalytic domain of full-length ROS-GC 1.

Experimenteller Teil

The catalytic subdomain of ROS-GC 1 containing an N-terminal lipoyl domain fusion tag (HLT) was expressed in E.coli and purified by Ni-affinity chromatography. After removal of the tag, the monomeric domain was obtained by size-exclusion chromatography. The enzymatic activity of the purified domain was evaluated by a functional cGMP assay using LC-SRM-MS to quantify enzymatically synthetized cGMP. Cross-linking analysis between the catalytic domain of ROS-GC 1 and GCAP-2 were conducted in the absence and presence of calcium using the amine-reactive isotopically labeled cross-linker BS2G-D0/D4, the amine-reactive MS/MS-cleavable urea-linker, the amine-/photo-reactive cross-linker SDA, and the zero-length cross-linker DMTMM. Proteolyzed reaction mixtures were analyzed by nano-HPLC/nano-ESI-MS/MS using an Orbitrap Fusion mass spectrometer. Cross-links were identified by the StavroX software.

Ergebnisse und Diskussion

In a previous study, cross-linking MS analysis between GCAP-2 and a peptide derived from catalytic domain of ROS-GC 1 pointed to a well-defined structure of the GCAP-2-GC peptide complex in the calcium-bound state, while results obtained in the absence of calcium indicated a high flexibility of calcium-free GCAP-2 in the complex with the GC peptide [1]. To gain more detailed insights into the mechanism of ROS-GC activation by GCAP 2 under more physiological conditions, we used the purified catalytic subdomain of ROS-GC 1 for cross-linking/MS analysis with GCAP 2. To test for the correct folding of the catalytic domain after expression in E.coli and purification, the enzymatic turnover of GTP to cGMP was followed by LC-SRM-MS and the enzymatic activity of the expressed domain was confirmed. Increasing turnover rates after addition of GCAP 2 indicate the functional interaction of the catalytic ROS-GC domain and GCAP-2, thus allowing us to use this system to study the native interaction of the two proteins. Cross-linking/MS analysis of the ROS-GC catalytic domain and GCAP-2 in the absence and presence of calcium lead to the identification of a number of intermolecular cross-linking products between ROS-GC and GCAP-2, which will allow to build a functional model for the interaction of these proteins.

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Structure analysis of Shiga toxin subtype 2a from the enterohemorrhagic Escherichia coli (EHEC) strain O111:H- by nanoESI mass spectrometry

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Keywords: Shiga toxin, subtype 2a, nanoESI mass spectrometry, sequence, molecular weight

Einleitung

Shiga toxins (Stxs) are members of the AB5 toxin family and are released by Stx-producing E. coli (STEC). Enterohemorrhagic E. coli (EHEC), a subset of STEC, besides severe diarrhea and hemorrhagic colitis give rise to extraintestinal complications like the life-threatening hemolytic uremic syndrome (HUS). After binding of the B5 homopentamer to the genuine receptor globotriaosylceramide (Gb3Cer) on the host cell surface and subsequent internalization the A subunit of the toxin reaches the ribosomes where the cleaved A1 fragment irreversibly inhibits protein biosynthesis. In the present study we demonstrate the mass spectrometric characterization of Stx2a from the EHEC strain O111:H-.

Experimenteller Teil

Stx2a was isolated from the culture supernatant of EHEC O111:H- by affinity purification. After SDS-PAGE separation the protein bands corresponding to A and B subunit were in-gel digested by use of trypsin. The proteolytic peptides were extracted and purified. MS experiments were performed by use of Synapt GS2 instrument in the positive ion sensitivity mode. Peptide sequences were deduced from fragment ion spectra derived from nanoESI ion mobility spectrometry (IMS) low energy CID experiments. Molecular weights of the subunits were obtained from MS analysis of the intact protein dissolved in 50 % aqueous methanol containing 5 % formic acid.

Ergebnisse und Diskussion

Coomassie staining of the SDS-PAGE of affinity purified Stx2a revealed two bands at 35 kDa (A subunit) and 8 kDa (B subunit), respectively. The bands were subjected to in-gel tryptic digestion, the proteolytic peptides were analyzed by nanoESI IMS low energy CID, and the sequences were determined from the resulting fragment ion spectra. For the A subunit 41.4 % and for the B subunit 100 % of the data base stored sequences were covered. Moreover, the disulfide bond between C22 and C75 of the B subunit was unambiguously proven by the observed characteristic fragmentation of the doubly charged peptide ions at m/z 1191.98 [1]. MS analysis of the intact protein revealed an average molecular weight of 32906.78 Da for the A subunit which is in agreement with the calculated value of 32907.24 Da corresponding to the mature protein missing the first two N-terminal amino acids. For the B subunit 7810.66 Da (monoisotopic) was found which fits perfectly with the expected calculated value of 7810.6813 Da.

The present study demonstrates that the combination of determination of molecular weights and peptide sequencing offers a fast and facile method for structure analysis of Stxs on protein level and the detection of polymorphisms of different variants of Stx subtypes [2].

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Synthesis of Less Hydrophobic Derivatives of MS/MS-Cleavable Cross-Linkers: Synthesis and Evaluation of Reactivity for Effective Protein Structure Analysis

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Keywords: Peptide Cross-Linking, (CID)-ESI-MS/MS, Protein structure analysis.

Einleitung

Chemical cross-linking (XL) in combination with mass spectroscopy (MS) has matured into a valuable alternative strategy for deriving structural information of proteins and protein assemblies, where NMR and X-ray crystallography reaches limits.

Several amine reactive homobifunctional N-hydroxysuccinimide (NHS) active ester reagents have been developed. A number of them come with MS/MS labile covalent bonds allowing sensitive collision-induced dissociation (CID)-analysis.However, the limited solubility of NHS-reagents in polar solvents is a bottleneck for extensive protein derivatization. To overcome this limitation we synthesized N-hydroxysulfosuccinimide (S-NHS) analogs of our NHS-cross-linkers. The cross-linking efficiency as well as the applicability in various buffer-systems are under investigation.

Experimenteller Teil

All experiments were performed with our well investigated "Urea-Linker"[1] and also with the very hydrophobic "Azo XL Linker".[2]

Introduction of the S-NHS-group was performed employing several carbodiimide-mediated peptide coupling reagents e.g. DIC, HATU, PyBOP, EDCI in combination with additives like HOBt, DIPEA, 4-DMAP and sym-collidine. The mixtures were analyzed by ESI-MS and NMR.

First cross-linking experiments were performed in HEPES-buffer using model-peptides (MRFA and Thymopentin). The CID-MS/MS experiments were carried out on an LTQ-Orbitrap XL instrument (Thermo Fischer Scientific) equipped with a heated ESI source.

Ergebnisse und Diskussion

In presence of pyridine-bases, the introduction of the S-NHS-group could be achieved with most coupling reagents. In general, the use of carbodiimides with pyridine-bases yielded best results. With a combination of EDCI and sym-collidine close to 100% of the used di-acids were converted into di-S-NHS compounds. ESI-MS analysis for all other "working" combinations showed at least ¼ mono S-NHS-ester.

S-NHS analogs show a substantially improved solubility even in water, while they are nearly insoluble in most organic solvents. Additionally, a strong pH-sensitivity towards hydrolysis is observed. The S-NHS-analogs seem to hydrolyze within minutes at pH-values >7.5.

Due to these special properties an effective purification protocol for the EDCI/collidine synthesis could not be achieved so far. Therefore, cross-linking studies were performed with samples of the S-NHS-cross-linkers containing traces of the inactive EDCI-Urea.

First cross-linking studies were performed in HEPES-buffer, using model-peptides and the S-NHS-analogs, following known protocols[3]. CID-MS/MS experiments document, that the new compounds are able to introduce cross-links, just as the original NHS-cross linkers. Due to the high water solubility of the new analogs, use of DMSO, which can be problematic in protein cross linking experiments, is no longer necessary. Besides that, EDCI-Urea impurities seem to have no influence on the reaction.

Furthermore, the ability to introduce cross-links in proteins and in other buffer-systems are under investigation.

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Targeted Mass Spectrometry based quantification of MYDGF in blood from patients suffering from coronary heart diseases

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Organisation(en): Medizinische Hochschule Hannover, Deutschland

Keywords: MYDGF, MRM, myocardial infarction, regenerative medicine

Einleitung

A new approach for the therapy of myocardial infarction (MI) includes the application of bone marrow cells into the heart which stimulate the heart regeneration. The beneficial effect is based on paracrine acting of secreted proteins [1]. We suggest a Myeloid-derived growth factor (MYDGF) to be a key player in regulation of cardiac myocyte survival and proliferation [2]. A therapeutic MYDGF application leads to significantly smaller infarct area and to improved survival rate of mice after MI. We established a mass spectrometry based multiple reaction monitoring (MRM) assay for the specific detection and quantification of plasma MYDGF for mice and human patients which may give insight on the correlation of plasma MYDGF with heart function-loss and a following therapy.

Experimenteller Teil

Based on Orbitrap-MS data, specific peptides were selected for MRM. Using recombinant protein a transition list was created and the collision energy and declusting potential were optimized. Blood plasma samples were taken from patients with a treated or untreated coronary heart disease and from patients with an acute MI. Additionally murine plasma from Sham and infarcted mice was analyzed. Plasma proteins were separated via SDS-PAGE and digested with Trypsin. For absolute quantification, peptides labeled with heavy amino acids were spiked-in to each sample at a concentration of 0.1 fmol/µl.The mixture was separated by a reverse phase high pressure liquid chromatography (RSLC, Thermo Fisher Scientific) and sprayed into a triple Quadrupol-MS (ABI4000, SCIEX). Generated data were analyzed with the Skyline-software.

Ergebnisse und Diskussion

We established a specific and sensitive assay for MYDGF with the use of Quadrupol-MS based MRM. The two peptides SYLYFTQFK (quantifier) and LVIVAK (qualifier) serve as surrogates for the abundance of the full length protein. The spike-in withsequence identical peptides labeled with heavy amino acids serves as an internal standard and allows the absolute quantification. With this assay we could quantify MYDGF up to a concentration of 100 amol in a matrix-free background and 6 fmolspiked in plasma samples from MYDGF-deficient mice.

Plasma samples from control patients with a stable coronary heart disease (control) were compared with plasma samples from patients 6 hours after myocardial infarction (MI). A significant increase ($p<0,005$) was observed. The result could be reproduced in the mouse model by comparing plasma of operated mice (sham) with plasma of mice after experimentally induced MI ($p<0,03$).The elevated plasma level of MYDGF in MI patients promotes its role as a biomarker and potential therapeutic target.

To prove that the sources of MYDGF are primary bone marrow cells, plasma samples from bone marrow transplanted mice were analyzed. By transplanting WT bone marrow into KO mice, the MYDGF plasma level could be recovered.

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The Adaptive Proteome of Pseudomonas aeruginosa

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Keywords: Pseudomonas aeruginosa, proteome analysis, label free quantification

Einleitung

The opportunistic pathogen Pseudomonas aeruginosa can cause very acute infections, particularly in the immunocompromised host. Chronic infections e.g. in cystic fibrosis patients are associated with bacterial growth within biofilms. Here, the bacteria are not only more resistant to antibiotics but they are also protected from the host immune system. The high adaptability to various environmental conditions is a characteristic feature of the opportunistic pathogen P. aeruginosa. This is reflected in adaptive transcriptional responses [1]. In this study we aimed to uncover the adaptive proteome of P. aeruginosa and to evaluate how this relates to the transcriptome.

Experimenteller Teil

P. aeruginosa PA14 was cultivated under different environmental conditions. Cells were lysed and extracted proteins separated by SDS-PAGE. In-gel digestion with trypsin was used and peptides were separated by reversed phase HPLC and measured via data-dependent analysis in an Orbitrap LTQ velos (Thermo Scientific) mass spectrometer. The open source software MaxQuant was used for protein annotation and label free quantification. Differences in protein abundances were assumed to be significant with a fold change of 2 and a p-value of 0.05.

Ergebnisse und Diskussion

Comprehensive proteome analyses were conducted to characterize changes in the P. aeruginosa proteome. Cells were harvested at exponential phase, mid logarithmic phase, stationary phase and after low osmolality or iron starvation conditions. In total 4042 proteins could be quantified representing 68 % of all known and predicted proteins of strain PA14. Out of these 75 % (3046) were quantified under all measured conditions. Differences in protein abundance were determined between the various conditions and found to be particularly strong when comparing the proteome in different growth phases. Additionally to proteome comparisons the differences between transcriptome and proteome were determined. These differences are expected to occur due to posttranscriptional regulation or protein/mRNA stability.

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Posternummer: 46

The phosphorylation of human αS1-casein: New, old and fake sites

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Keywords: Human alpha S1 casein, phosphorylation, MRM, quantification

Einleitung
Human αS1-casein (CSN1S1) is the casein variant on which the least information is available (~5% of the casein fraction in breast milk). It was shown to exhibit pro-inflammatory properties, to skew in vitro differentiation of monocytes towards a macrophage-like phenotype and it turned out to be an autoantigen. We previously determined the CSN1S1 content in breast milk (1st week post partum, 3-540 µg/ml). Phosphorylation seems to be important, but information is scarce. Therefore, we identified and quantified phosphorylation sites of expressed CSN1S1 and protein isolated from breast milk. (see [1] and references therein)

Experimenteller Teil
Breast milk samples were treated as described [1]. Human embryonic kidney cells HEK-293 were transfected and Escherichia coli cells were transformed with plasmid constructs in order to overexpress CSN1S1 for purification [2]. E. coli-expressed protein was phosphorylated using human protein kinase CK2. Samples were separated by 1D-PAGE and CSN1S1 bands were subjected to proteomic analysis as before [1]. PseudoMRM was performed on non-, mono- and diphosphorylated tryptic peptides using Q-TOF Premier or Synapt G2 Si with nanoLC (nanoAcquity or M-Class), all of Waters Corp., for specific detection and quantification based on fragment ion intensities.

Ergebnisse und Diskussion
Two tryptic peptides (27LQNP<u>S</u>E<u>S</u>SEPIPLE<u>S</u>R42, 83MES<u>S</u>I<u>SSSS</u>EEMSLSK98; P47710-1) are known to be phosphorylated. In breast milk, we confirmed single and found considerable double phosphorylation at S33 and S41 (50% and more). Marker fragment ions also evidence phosphorylation of S89. This peptide was harder to detect in some human samples than in expressed protein where S33 and S89-containing phosphopeptides were measured with ~10-20% of the intensity of the unmodified peptide. In milk, S71 was shown to be phosphorylated in peptide 69NE<u>S</u>TQNCamVVAEP82 (<10%); a site only suspected so far by comparison to the bovine homologue. A pseudo-MRM quantification method was developed based on the masses of the doubly-charged ions of modified and unmodified peptides 27-42, 69-82, and 83-98.

On a side note, we report the false discovery of Y109 as potential phosphorylation site. It had been assigned with high confidence from data of different samples, instruments, and algorithms. Fragment ions matched perfectly the expected y-ion series after loss of phosphate. However, close manual inspection of the MS-scans revealed hyperoxidation of the peptide ion (no RP-separation; multiple mass shifts of 16 Da – 5x16 mimicking addition of 80 Da). We have observed this phenomenon also in unrelated samples and suspect the electrospray process as being the culprit for the undesired adduct formation. Such artefacts are not caught in untargeted analyses. They cause false assignments of phosphorylation sites and may trigger further resource-consuming research efforts.

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The Proteome of Human Cells Is Altered by Toxins of Clostridium difficile

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Keywords: Clostridium difficile, TcdA, SILAC, proteomics

Einleitung
The anaerobe Clostridium difficile is one of the most common nosocomial pathogens and triggers antibiotic-associated gastrointestinal infections ranging from mild diarrhea to life-threatening pseudomembranous colitis. Toxins TcdA and TcdB are the two major virulence factors of C. difficile that specifically glucosylate and inactivate small GTPases. The consequences are reorganization of the cytoskeleton, loss of cell-cell contacts, and finally cell death.

Experimenteller Teil
Comprehensive proteome analyses were conducted using data-dependent shotgun proteomics and human epithelial cell lines that had been treated with wild type or mutant toxins. Protein quantification was done by the SILAC technique. Long (24 h) and short term (8 h) effects of TcdA and TcdB on Caco-2 and Hep-2 cells were analyzed. Proteins were analysed by gel-LC-MS and results were verified by western blot and MRM analyses.

Ergebnisse und Diskussion
Wild type toxins induced considerable changes in the protein profile of epithelial cells. In case of TcdA more than 800 proteins of the over 6000 identified proteins were altered in their abundance. Regulated proteins were primarily involved in regulation, metabolic processes, endocytosis, organelle function, cell-cycle and cytoskeleton organisation. Glucosyltransferase deficient TcdA induced only changes after short incubation periods. After treatment with TcdB 183 of 5320 identified proteins were affected. These proteins are involved in signal transduction, cytoskeleton organisation, cell-cycle and cell death. The mutant TcdB revealed a pyknotic effect, which was glucosyltransferase independent but triggered the response of 129 proteins. Besides identified targets of TcdA, glucosylation was additionally identified in Rap1(A/B), Rap2(A/B/C), Ral(A/B), and (H/K/N)Ras, which had not been identified as TcdA targets so far.

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Posternummer: 48

Towards systematic benchmarking of siRNA delivery systems by relative protein quantitation

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Keywords: RNA interference, mTRAQ

Einleitung

Inhibiting specific gene expression by small interfering RNA (siRNA) bears broad potential in basic biological science and therapeutic applications [1]. As siRNA on its own is not able to cross the cellular membrane, effective measures to transport siRNA into cells are required. In the most promising approaches, siRNA molecules are encapsulated in nanoparticles made of lipids, peptides or cyclodextrin polymers which can readily enter mammalian cells. After the siRNA is incorporated into the RNA interference machinery, the complementary messenger RNA (mRNA) is cleaved in multiple rounds.

Experimenteller Teil

The efficiency of siRNA delivery and the subsequent gene silencing process are characterized by the remaining amount of the targeted mRNA and its protein translation product, respectively. mRNA quantification via real-time quantitative PCR (qPCR) does not necessarily reflect the protein level and additionally may be error-prone [2]. On the other hand, protein quantification via western blot is frequently hampered by the availability of specific antibodies. Hence, we intend to systematically compare different siRNA delivery systems by quantifying not only the target mRNA molecules via qPCR but also the target proteins via mass spectrometry. To this end, the proteins of interest are relatively quantified after labeling with mTRAQ (mass differential Tags for Relative and Absolute Quantitation) reagents.

Ergebnisse und Diskussion

In contrast to the usual labeling step at the peptide level, mTRAQ labeling is performed at the protein level. As a consequence, samples are combined at the earliest possible stage of the protocol, thus reducing measurement uncertainty.

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What’s in the Gift? Towards a Molecular Dissection of Nuptial Feeding in a Cricket

Autoren: Pauchet, Yannick (1); Wielsch, Natalie (2); Wilkinson, Paul A. (3); Sakaluk, Scott K. (4); Svatoš, Aleš (2); Ffrench-Constant, Richard H. (3); Hunt, John (3); Heckel, David G. (1)

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Keywords: nuptial gifts, LC-MS/MS, homology-based proteomics

Einleitung
Nuptial gifts produced by males and transferred to females during copulation are common in insects. Yet, their precise composition and subsequent physiological effects on the female recipient remain unresolved. Male decorated crickets Gryllodes sigillatus transfer a spermatophore to the female during copulation that is composed of an edible gift, the spermatophylax, and the ampulla that contains the ejaculate. In this study we investigated the protein composition of G. sigillatus spermatophylaxes using proteomics. To facilitate protein identification, we coupled proteomics analysis to the transcriptome sequencing of male accessory glands, the tissue producing spermatophylaxes in male G. sigillatus.

Experimenteller Teil
A normalized cDNA library from accessory glands dissected from sexually active male G. sigillatus was prepared using 454 pyrosequencing. The resulting transcriptome was assembled in contigs which were subsequently translated in the six possible open reading frames (ORFs) and compiled into a protein database that was then used for the proteomics analyses.

Proteins were extracted from lyophilized spermatophylaxes and separated by anion exchange chromatography (first dimension of separation). Each protein fraction was then resolved on a gradient (4–12%) SDS-PAGE gel (second dimension) and stained with colloidal Coomassie blue. Protein bands were recovered from the gel, tryptically digested and analyzed by LC-MS/MS. The acquired spectra were sequenced de novo and searched using MS-BLAST program for homology-based protein identification [1].

Ergebnisse und Diskussion
Twenty protein bands were recovered and analyzed by LC-MS/MS. De novo sequenced peptides were first searched against the Insecta subdivision of the GenBank non-redundant protein database. We were unable to identify a single spermatophylax (SPX) protein. De novo peptide predictions were then searched against the subset “Orthoptera” of InsectaCentral, a central repository of insect transcriptomes. Three SPX proteins having a high degree of similarity with other orthopteran-derived proteins, namely a chitinase-like, a fasciclin-like and a transferrin-like protein were confidently matched.

Searching the acquired peptides against the newly created G. sigillatus protein database allowed us to link the majority (17 out of 20) of these proteins to individual sequences present in the accessory gland transcriptome. To nine out of the 17 matched proteins no homologs could be found in model insects. Among the eight G. sigillatus proteins were a serine protease inhibitor harboring two pacifastin domains, an odorant-binding protein, a chitinase-like protein also annotated as a “wing disc growth factor-like protein”, a serine carboxypeptidase, two carbonic anhydrases, a fasciclin-like protein and a protein with similarity to an accessory gland protein derived from other cricket species.

<u>Conclusion:</u> spermatophylaxes contain proteins, some of which are similar to known insect proteins and some are novel. Protease inhibitors targeting trypsins and chymotrypsins might protect SPX proteins from proteolysis by proteases present in the digestive tract of female crickets. Presence of a potential polypeptide growth factor could promote cell growth and development in its target tissue within the female’s body or possibly influence female reproduction [2].

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µLC-ESI HR MS/MS method for bioanalytical verification of exposure to V-type nerve agents by simultaneous detection of diverse albumin-adducts

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Keywords: nerve agent, disulfide adduct, high-resolution mass spectrometry

Einleitung

Chemical warfare agents, among which the V-type nerve agents VX, Chinese VX (CVX) and Russian VX (RVX) belong to the most toxic artificial substances, are banned by the Chemical Weapons Convention. Therefore, verification analysis providing unambiguous evidence for an alleged use of such substances plays an important role with great political and legal impact. As nerve agents exhibit quite short half-lives in vivo, there is a great need for long-term biomarkers like specific protein-adducts. Due to the high abundance of human serum albumin (HSA) in plasma and its high stability in vivo, methods were developed for identification and detection of adducts between V-type nerve agents and HSA [1].

Experimenteller Teil

Following incubation of HSA at physiological concentration and human plasma with V-type nerve agents in vitro the samples were subjected to washing steps for removal of excess nerve agent. Using pronase, a mixture of several endo- and exopeptidases isolated from Streptomyces griseus, proteins were degraded. Resulting cleavage products were separated by microbore high-performance liquid chromatography (µLC) and detected online by modern high-resolution tandem-mass spectrometry (HR MS/MS) after positive electrospray ionization. For dose-response studies different molar ratios of nerve agents:HSA were tested allowing estimation of limits of detection.

Ergebnisse und Diskussion

In addition to already known phosphonylated tyrosine residues [2] novel disulfide adducts were identified, that had been formed between the thiol containing leaving group of the respective V-type nerve agent and the cysteine-proline dipeptide (Cys34-Pro, CP). Structural elucidation was done by detecting the exact mass of precursor ions and their fragments derived from collision-induced dissociation (CID). Simultaneous separation and detection of both tyrosine- and CP-adducts, required elevated column temperature to minimize peak broadening of CP caused by its cis/trans isomerism [3]. Dose-response studies of CP-adducts from human plasma in vitro showed sigmoid curvatures with a plateau at high excess of V-agents indicating quantitative derivatization of free cysteine residues. However, linearity of peak areas was found at low, toxicologically relevant concentrations of the V-type nerve agents for both adducts. CVX revealed highest reactivity when compared to RVX and VX. For all V-agents limits of detection were found at 118 nM for the CP-adducts as well as for the tyrosine-adduct of VX. RVX revealed 294 nM and CVX 588 nM for their respective tyrosine-adducts. Therefore, this method might represent a valuable and novel supplement of existing methods for verification.

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Characterization of human protein kinase D2 complexes by chemical cross-linking and mass spectrometry

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Keywords: Protein kinase D2, Affinity enrichment, Cross-Linking, Label-free quantification

Einleitung

Protein kinase D (PKD) is a serine-threonine kinase comprising three isoforms, which is involved in various functions of eukaryotic cells, such as cytoskeleton dynamics or protein transport. As a particular example, PKD2 is supposed to be involved in the fission of transport vesicles at the trans-Golgi network (TGN) [1]. In order to identify underlying protein-protein interactions and to map the interface regions between PKD2 and interacting proteins, we applied chemical cross-linking and mass spectrometry [2].

Experimenteller Teil

For identifying PKD2 interaction partners, an affinity enrichment approach in combination with chemical cross-linking was employed. For this, affinity-immobilized GST-tagged PKD2 was incubated with cytosolic protein fractions and Golgi-enriched protein fractions obtained from subcellular fractionation of HeLa cells. After chemical cross-linking with the homobifunctional amine-reactive reagent BS2G-D0/D4 protein complexes were enzymatically digested with Glu-C and trypsin and analyzed by nano-HPLC/nano-ESI-Orbitrap mass spectrometry (UltiMate 3000 RSLCnano/Orbitrap Fusion, Thermo Scientific). Identification and label-free quantification of proteins was carried out with the MaxQuant software and statistical evaluation using the Perseus software [3].

Ergebnisse und Diskussion

With the affinity enrichment/cross-linking strategy, a number of potential interaction partners of PKD2 were identified, which were captured exclusively in cross-linked samples. These most likely represent weak or transient interactions demanding fixation by the cross-linking step. One target identified in cytosolic fractions is the seven-subunit Arp2/3 complex, which is a major component of the actin cytoskeleton regulatory machinery involved in cytoskeletal dynamics and vesicle motility. Remarkably, all seven subunits of the Arp2/3 complex were recovered in the cross-linked samples.

Currently, the PKD2-Arp2/3 interaction is subjected to a more detailed structural characterization by further cross-linking experiments between PKD2 and the purified Arp2/3 complex using cross-linking reagents with complementary reactivies.

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Effect of a complex environmental stress on Arabidopsis thaliana proteome and metabolome in context of protein glycation

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Keywords: AGEs, glycation, post-translational modifications, tandem mass spectrometry, plant environmental stress

Einleitung

Drought and light are the major environmental factors reducing world crop productivity. These factors trigger metabolic adjustment (e.g. enhanced generation of sugar metabolites) and increase of reactive oxygen species (ROS) generation. The combination of these alterations provides the ideal metabolic background for protein glycation, and might enhance accumulation of advanced glycation end-products (AGEs). These compounds are toxic and pro-inflammatory in humans. Despite of this, stress-specifically glycated plant proteome is not characterized so far. Therefore, here we report the dynamics of stress-related changes in the patterns of protein expression and advanced glycation in the Arabidopsis thaliana model of combined drought/light stress.

Experimenteller Teil

The agar-based polyethylene-glycol (PEG) infusion drought model was established with six week-old A. thaliana and validated by a panel of physiological and biochemical markers. For metabolomic and proteomic experiments, the leaves were harvested before and at 1 – 10 days after the stress application. Metabolite profiling relied on untargeted GC-EI-Q-MS experiments. The proteome was analyzed by the LC-based bottom-up proteomics approach in LC x LC-ESI-Orbitrap-LIT-MS/MS data dependent acquisition experiments. The data were interpreted by Progenesis software. Individual glycation sites were quantified by label-free techniques. The changes in protein patterns and functional annotation of differentially expressed/glycated proteins relied on bioinformatic tools and systems biology approach.

Ergebnisse und Diskussion

A significant decrease in stomatal conductance, increase in malondialdehyde, abscisic acid, proline, and sucrose contents clearly demonstrated development of drought stress. In the combined stress experiment 245, 432, and 529 differentially expressed proteins were identified after 3, 7, and 10 days upon drought application, respectively. Already after three days of treatment, 44 drought specific proteins were found to be stress-specifically AGE-modified, while the levels of advanced glycation changed in 29 proteins. The AGE modifications dominated with glyoxal-derived species that was in agreement with observed α-dicarbonyl patterns. The functional annotation of the stress-affected proteins revealed those related to protein metabolism and regulation of transcription as the most affected groups. However, dehydrins and polypeptides involved in late embryogenesis were over-expressed as well.

Referenzen

Dissociation behavior of a bifunctional TEMPO-benzyl active ester used for peptide structure analysis by free-radical-initiated-peptide-sequencing(FRIPS) mass spectrometry in negative ESI-MS

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Keywords: (-)-ESI-MS, peptide-cross-linking, free-radical-initiated-peptide-sequencing

Einleitung

We synthesized a cross-linker containing a TEMPO (2,2,6,6-tetramethylpiperidine-1-oxy) moiety connected to a benzyl group (Bz), termed TEMPO-Bz-linker.[1]The aim for designing this novel cross-linker was to facilitate MS analysis of cross-linked products by free radical initiated peptide sequencing (FRIPS) and to evaluate the potential of the TEMPO-Bz-linker for protein 3D-structure analysis. Previous (+)-ESI-MS/MS studies had revealed an unexpected fragmentation behavior of the TEMPO-Bz-linker upon collisional activation following two distinct fragmentation pathways, depending on charge state and amino acid sequence of the peptides involved.[1,2](-)-ESI-MS, MS/MS, and MS^3 experiments of cross-linked peptides allowed elucidating the fragmentation behavior of deprotonated TEMPO-Bz species.

Experimenteller Teil

Angiotensin II and ACTH (fragment 18-39) were purchased from Sigma-Aldrich.

Test peptide 1 (Ac-TRTESTDIKRASSREADYLINKER) was obtained from Creative Molecules Inc. The TEMPO-Bz-linker[1] (1 mM in DMSO) was added to the peptide solution in 20 mM HEPES, pH 8.5 (cross- linker peptide- ratio 1:1). The reaction was allowed to proceed at 25°C for 2 hours, before cross-linked test peptide 1 was digested with trypsin. All other crosslinked peptides were used directly. (-)ESI-MS, MS/MS, and MS^3 analyses were performed on an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific). For MS/MS measurements, ions were isolated in the quadrupole, fragmented by CID and analyzed in the orbitrap mass analyzer (R = 120,000 at m/z 200).

Ergebnisse und Diskussion

Cross-linking of three model peptides was performed with the MS/MS cleavable TEMPO-Bz-linker. For elucidating the fragmentation behavior of the cross-linker, (-)-ESI-CID-MS/MS and MS^3 experiments were performed. Data analysis was performed using the in-house MeroX software[3] and by manual assignment of FRIPS fragments and neutral losses. (-)ESI-MS3 spectra were collected to examine the different fragmentation behavior of TEMPO- and Bz-modified peptide chains. Side-chain fragmentation was preferentially observed for Bz-modified peptides, while backbone fragmentation was dominant for TEMPO-modification. The crosslinked, 3- charged precursor ion of test peptide 1 fragmented as expected with a homolytic cleavage at the NO-C bond of the TEMPO-Bz- linker. (-)-ESI-MS/MS indicated a large degree of backbone fragmentation of peptides (a, x, c, y-type ions). The 2- charged precursor ion of intermolecularly crosslinked angiotensin II showed the expected homolytic fragmentation of the TEMPO-Bz-linker. Interestingly, the loss of one intact peptide chain was the dominant fragmentation pathway. For the 3-charged precursor ion of the intermolecularly cross-linked more acidic peptide ACTH, peptide losses were exclusively observed. Assigning all fragments in (-)-ESI-MS, MS/MS, and MS^3 was challenging due to the different fragmentation pathways of TEMPO-Bz-crosslinked peptides. However, the expected homolytic cleavage of the cross-linker was observed in most cases.

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Skin Transplant Quality Assessment by Multiplexed 2D MRM Mass Spectrometry

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Keywords: Multiple reaction monitoring (MRM), Plasma protein quantitation, Transplant quality assessment

Einleitung
Free flap transplantation has become the gold standard to cover injury-derived skin-less body parts. However, as inspection of clinical signs in case of post-transplantation complications may come too late, loss of transplants may result. Hence, monitoring of tissue parameters, to assess transplant quality earlier than by clinical means, is needed [1]. Differential protein expression is expected to carry information on the free flap´s status by which its quality can be assessed. We collected human plasma samples during free flap transplantation surgery from the feeding artery and the effluent vein to study their respective plasma protein profiles by 2D LC/MRM-MS [2].

Experimenteller Teil
Proteins from 20 µl of plasma were denatured, reduced and alkylated. After digestion with trypsin, 50 stable-isotope-labelled standard peptides (SIS peptides) were spiked-in. Peptide mixtures were desalted and concentrated by solid phase extraction. After washing, peptides were eluted with 600 µl 55% ACN in 0.1% FA and lyophilized. For injection into the high-pH RPLC system, peptides were rehydrated with 1600 µl 10mM ammonium hydroxide solution. Upon LC fractionation of the peptide mixture into 47 wells of a 96 well plate, eluates were lyophilized. Before analysis with low-pH RPLC/MRM-MS, neighboring fractions were combined, generating 13 fractions for each sample in 100 µl of 0.1% FA, each. Peptides were detected on a QqQ mass spectrometer (Agilent 6490).

Ergebnisse und Diskussion
From twenty-one patients in our study seven were clinically diagnosed with free flap complications after surgery. A panel of 50 proteotypic peptides, representing 24 plasma proteins belonging to inflammatory, coagulation, and acute-phase proteins were spiked-in into each digested plasma sample (3 samples per patient). Counting all MRM transitions, a total of 9450 data points were acquired. After quality control of all MRM assays, 21 proteins (8742 data points) were quantified in each plasma sample, spanning five orders of magnitude. Inter-patient coefficients of variances (CV%) ranged from 26 for complement C3 to 189 for serum amyloid A 1 protein.

Maximum Youden index analysis for macrophage colony-stimulating factor (MCSF) indicated that the optimal threshold for discriminating complications from controls was 21.33 ng/ml. The receiver operator characteristic (ROC) analysis for MCSF resulted in an area under curve (AUC) of 0.841, which is of clinical value. Furthermore, to test which statistical significance is reached with our assay, a power analysis was performed and provided the actual power of 0.96 and the minimally required sample size of N = 24, which is well met by our patient cohort.

MCSF is the major macrophage growth factor that has been reported to increase during tissue inflammation [3]. Our study proves that plasma levels of MCSF were elevated in the free-flap complication group as compared to control group, suggesting MCSF as potential early biomarker to evaluate the risk of free flap complications at an early stage, leaving time for surgeons to initiate salvaging interventions.

Referenzen

Mass spectrometric characterization of ezrin structures in breast tumors and lymph nodes – on the way towards a molecular metastasis marker

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Keywords: sentinel lymph node, breast cancer, metastasis marker, ezrin, mass spectrometric protein structure characterization

Einleitung
Metastases are in most cases the cause of death in breast cancer patients. At time of primary tumor surgery metastasis status of the axillary lymph nodes is an important clinical indicator of prognosis for survival [1]. At current lymph node status is determined by time-consuming pathological means requiring skilled specialists. To assist objective status determination with automation potential, a molecular marker was desired. Ezrin participates in metastasis of breast cancer cells and its expression is estrogen regulated [2]. We investigated ezrin expression in breast tumors, healthy breast tissue, axillary lymph nodes and sentinel lymph node metastases and determined truncation patterns of ezrin by mass spectrometric protein structure analysis in order to provide a molecular marker for lymph node metastases.

Experimenteller Teil
Primary tumors (n=8), healthy breast tissues (n=8), axillary lymph nodes (n=10) and sentinel lymph node metastases (n=12) were taken from breast cancer patients. Protein extracts were subjected to 1D-gel electrophoresis (35 µg/sample). Ezrin expression was detected by Western blotting using a monoclonal antibody against the C-terminal region (Pierce Biotechnology). Gel bands were excised and tryptically digested. Precursor ion masses and associated fragment ions were measured in an alternate scanning mode (MSE) with a SYNAPT G2-S HDMS mass spectrometer coupled to a nanoUPLC (Waters) [3]. Protein identifications were accepted if at least five fragment ions per peptide, ten fragment ions per protein and two peptides per protein were determined. Additionally, each fragment ion spectrum assigned to ezrin underwent manual assessment.

Ergebnisse und Diskussion
Western blotting and staining with the ezrin antibody (aa 362-585 were used as immunogen) revealed that full-length ezrin (form E1), migrating at ~90 kDa (top band), was present in primary tumors, healthy breast tissue, axillary lymph nodes and sentinel lymph node metastasis samples. Additionally, a truncated ezrin form (form E2) migrating at ~65 kDa (middle band) was present in tumor tissue and sentinel lymph node metastases. Interestingly, the sentinel lymph node metastasis samples showed another truncated ezrin form (form E3) migrating at ~50 kDa (bottom band).

In-depth MS and MS/MS measurements of 1D- gel bands confirmed the presence of the full-length protein (form E1), with a theoretical mass of ~70 kDa, in the top band. Peptide mapping of the middle band (form E2) revealed a major ezrin fragment missing the first hundred N-terminal amino acids with a theoretical mass of ~60 kDa. Surprisingly, one metastasis sample showed a C-terminally truncated ezrin form, covering aa 1-435 with a theoretical mass of ~50 kDa in this middle band. MS analysis of the bottom band (form E3) indicated the presence of a C-terminal ezrin fragment covering amino acids 238-586 with a theoretical mass of ~40 kDa. Of note, by mass spectrometry we identified an N-terminal fragment of ezrin (form E4), covering aa 1-273, that lacks the epitope region of the used antibody in a band at ~37 kDa.

In summary, mass spectrometric characterization explained the observed band pattern of ezrin in the investigated samples. Furthermore, the strong expression of the C-terminal ezrin fragment covering amino acids 238-586 exclusively in the sentinel lymph node metastasis samples predestines this ezrin form to become a molecular marker for lymph node metastases of breast cancer patients. The identification of such markers is a prerequisite for the basic understanding of molecular and cellular processes related with metastasis.

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Improved diagnostic accuracy for type 2 diabetes by combining glycation sites of plasma proteins with established diagnostic criteria

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Keywords: Biomarker, type 2 diabetes mellitus, multiple reaction monitoring (MRM)

Einleitung

Type 2 diabetes (T2D) is the most common form of diabetes accounting for 90-95% of all clinical cases. Current diagnostic criteria based on HbA1c-levels and fasting plasma glucose (FPG), identify only around one third of previously undiagnosed patients. Possessing a high sensitivity towards slightly elevated plasma glucose levels, glycated proteins have been recognized as potential markers of hyperglycemia. All methods established so far determine the overall glycation of proteins, although quantification of individual glycation sites in multiple proteins may provide better and earlier diagnostic measures.

Experimenteller Teil

Plasma samples obtained from newly diagnosed diabetic patients and non-diseased individuals were separated from low-molecular weight compounds, digested with trypsin, enriched for glycated peptides by boronic acid affinity chromatography (BAC), desalted by solid phase extraction (SPE), and separated by RP-HPLC coupled online to ESI-QqQ-MS. Quantification relied on multiple reaction monitoring (MRM) of multiple glycation sites identified in plasma proteins using a stable isotope dilution approach.

Ergebnisse und Diskussion

Based on our previous studies that identified 40 glycation sites as prospective biomarkers in a small number of plasma samples taken from T2D patients and healthy individuals (1), we selected and synthesized 30 peptides as standard compounds (2; 3). The most promising 19 peptides were also synthesized with one or two 13C,15N-labeled amino acids as internal standards. All steps from sample preparation to analysis, i.e. tryptic digestion, BAC, SPE, and RPC-MS, were optimized for recovery and precision and finally validated.

The plasma levels of 27 glycation sites corresponding to nine different plasma proteins were significantly increased (at least $P < 0.05$) in samples obtained from 48 male diabetic patients relative to 48 healthy individuals matched for age (35-65 years), body mass index (BMI), and gender. The peptides provided sensitivities of up to 79% and specificities of up to 88%. When the levels of a haptoglobin peptide were combined with HbA1c and FPG as current diagnostic criteria, the accuracies increased to 96% and 91%, respectively. Interestingly, glycation sites of plasma proteins followed other glycation kinetics than hemoglobin (i.e. HbA1c) indicating the potential of an additional diagnostic tool. The cluster analysis of the diabetic plasma samples revealed three as optimal number of clusters suggesting a possible subgrouping of the diseased subjects.

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A comparison of 2,5-dihydroxybenzoic acid and 9-aminoacridine as matrices for lipid analysis

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Keywords: MALDI-TOF MS, matrix, lipids, oxidation products

Einleitung

The selection of the most suitable matrix is a crucial step in MALDI MS to minimize interferences between matrix and analyte signals. For lipid analysis 9-aminoacridine (9-AA) has been suggested as a powerful matrix [1] providing important advantages: (a) 9-AA offers a higher sensitivity than other matrices such as 2,5-dihydroxybenzoic acid (DHB), (b) 9-AA favours the generation of H+ adducts and suppresses the generation of alkali adducts, (c) the background is moderate and (d) 9-AA is particularly useful for the negative ion mode. Here, some disadvantages of 9-AA with respect to the analysis of sperm lipids will be demonstrated.

Experimenteller Teil

All chemicals, solvents, lipids and matrices were obtained in the highest commercially available purity from Sigma-Aldrich (Taufkirchen, Germany) and used as supplied. Phospholipid standards were purchased from AVANTI Polar Lipids (Alabaster, AL, USA). DHB was used as 0.5 M solution in methanol, while 10 mg/ml 9-AA were dissolved in isopropanol/acetonitrile (60/40, v/v)) [1]. Spermatozoa of different species were investigated as physiologically relevant samples. Lipid extraction was performed by chloroform/methanol according to the procedure of Bligh and Dyer. All lipid standards and lipid extracts were pre-mixed with matrix (1:1, v/v) prior to application to an aluminum MALDI target. Positive and negative ion spectra were recorded using an Autoflex workstation (Bruker Daltonics GmbH, Bremen, Germany) in the reflector mode.

Ergebnisse und Diskussion

Cells and tissues contain a large variety of lipids. Besides zwitterions, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SM) or acidic phospholipids (phosphatidylinositol (PI)) there are also neutral lipids such as cholesterol and triacylglycerols. All lipid classes can be identified using DHB as matrix comparing the positive and negative ion mass spectra. However, for the detection of PE from lipid mixtures DHB is not recommended due to the suppression of PE signals by PC in the positive and PI in the negative ion mode. This problem can be overcome using 9-AA allowing the detection of PE as negative ions. Nevertheless, unequivocal identification of PE remains difficult due to detection of PC as negative ions in the presence of 9-AA. Triacylglycerols are not detectable using 9-AA as matrix.

During inflammation tissues and immune cells generate reactive oxygen species (ROS) such as HOCl or hydroxyl radicals [2]. This leads to oxidation of lipids resulting in a variety of lipid oxidation products such as peroxides and lysophospholipids (LPL). In addition to diacyl PL, sperm of several animal species contain alkenyl-acyl PL termed "plasmalogens” [3]. Plasmalogens contain an alkenyl ether linkage that is sensitive to oxidation resulting in the formation of LPL and formyl-LPL under oxidative stress conditions. Both are exclusively detectable with DHB but not with 9-AA as matrix. Although there is no convincing explanation for this phenomenon, we conclude that data from mass spectra of complex lipid mixtures need to be analyzed with caution.

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A Novel Lipid Screening Platform Allowing a Complete Solution for Lipidomics Research

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Keywords: lipds, lipidomics, ion mobility, metabolomics

Einleitung
A major challenge in lipid analysis is the many isobaric and near isobaric interferences present in highly complex samples that confounds identification and accurate quantitation. This problem, coupled with complicated sample preparation techniques and data analysis, highlights the need for a complete solution that addresses these difficulties and provides a simplified method for analysis. A novel lipidomics platform was developed that includes simplified sample preparation, automated methods, and streamlined data processing techniques that enables facile, quantitative lipid analysis. Herein, serum samples were analyzed quantitatively using a unique internal standard labeling protocol, a novel selectivity tool (differential mobility spectrometry; DMS) and novel lipid data analysis software.

Experimenteller Teil
A QTRAP® System with SelexIONTM Technology (SCIEX) was used for targeted profiling of over a thousand lipid species from 10 different lipid classes (Figure 2) allowing for comprehensive coverage. Two methods covered ten lipid classes using a flow injections analysis (FIA) included the following coverage; one injection with SelexIONTM Technology ON and another with the SelexIONTM Technology OFF. Lipid species were measured using MRM and positive/negative switching. Negative ion mode detected the following lipid classes – FFA/LPE/LPC/PC/PE. Positive ion mode detected the following lipid classes – SM/DAG/CE/CER/TAG.

Samples were quantitated using software accompanying the full solution which incorporates the novel labeled internal standards available as a kit (over 90internal standards across 10 classes), developed for this platform (Avanti Lipids).

Ergebnisse und Diskussion
It will be demonstated that this system allows for:

1. Quantitative results for each lipid class as a sum of individual species 2. Mol% composition was obtained computationally from lipid molecular species data 3. Accurate lipid species compositions. The data was compared with historical data generated by alternative methods.

The LipidyzerTM Platform fully elucidates the class and fatty acid composition of each lipid molecular species. Ten classes covering over 1100 species means a comprehensive coverage of complex lipid metabolism.

LipidyzerTM internal standards were compared to the use of a single internal standard (dCE(16:0)) for their ability to accurately calibrate the concentration of total cholesteryl esters and the fatty acid (A) composition of cholesteryl esters (B) in human serum. Twenty-five human serum samples with known total CE and CE fatty acid compositions were profiled using the LipidyzerTM Platform. The LipidyzerTM Platform quantified total CE with less than 10% bias, compared to a 100% bias in the estimate made using a single internal standard. The LipidyzerTM platform also removed the compositional bias obtained when using a single internal standard, providing an accurate fatty acid composition of CE.

Triglyceride data shows that 99.6% of total TAG class mass contributors have robust RSD's of 10% or less, with 88.3% of the individual TAG species that have a RSD of 20% or less.

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Development of a nanoLC-MS method for Lipidomics

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Keywords: NanoLC, Lipidomics, Method Development

Einleitung
Lipids are key players of cellular systems that are particularly known for their roles in structural compartmentalization and energy storage. Furthermore, they are also able to fulfill signaling functions in biological systems at marginal concentrations. In order to identify low abundant lipids such as mediators, analytical methods need to be developed to allow an accurate characterization and quantification of these biomolecules with high sensitivity and specificity.

Experimenteller Teil
In comparison to conventional LC-MS analyses, nanoLC-MS systems promise significant higher measurement sensitivity, reduced sample consumption, and decreased solvent usage. For this reason, we developed a nanoLC-ESI-MS method for lipid analyses.

Ergebnisse und Diskussion
Different column materials, flow rates temperatures and solvent compositions were compared to investigate their impact on nano-scale reverse phase chromatography. The reproducibility and robustness of the method was shown through repetitive separations of a yeast extract on the developed nanoLC-MS system.

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Enhanced IR-MALDI-MS Imaging of Crude Lipid Extracts from HPTLC Plates by Use of Ion Mobility Separation

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Keywords: HPTLC-IR-MALDI-MS, ion mobility, noise reduction, lipid analysis

Einleitung

The coupling of high-performance thin-layer chromatography (HPTLC) and mass spectrometry (MS) is a valuable tool for the analysis of lipid mixtures. Especially the direct analysis of HPTLC bands by infrared matrix-assisted laser desorption/ionization (IR-MALDI) enables a relatively fast and label-free measurement of separated phospho- (PL) and glycolipids [I,II]. However, the analytical sensitivity is often limited by chemical noise, originating from clusters of the IR-MALDI matrix (typically glycerol) and silica gel-derived background ions. Here we show that ion mobility separation (IMS) can be effectively used to separate analyte signals and chemical noise. The greatly enhanced sensitivity together with the additional drift time information were used for obtaining comprehensive lipid profiles and MS images from crude lipid extracts of monocytic THP-1-cells.

Experimenteller Teil

An IR-OPO-laser system tuned to 2.94 µm (GWU Lasertechnik; t=7 ns, frep=20 Hz) was coupled to a Synapt G2-S ion mobility mass spectrometer (Waters). Utilizing a beam shaping approach similar to the one described previously [III], an elliptical focal spot of about 35 µm x 50 µm was realized. Crude lipid extract of THP-1-cells was produced and chromatographed as described before [II]. Uniform soaking of the silica gel with the glycerol MALDI matrix was achieved with a new vacuum-assisted dipping method. MS images of the HPTLC plates were recorded at a step size of 100 µm. 20 laser shots were applied per pixel. Driftscope (Waters) and BioMap (Novartis) software were used for data processing.

Ergebnisse und Diskussion

Combining HPTLC separation, 2D-IR-MALDI MS imaging and IMS resulted in a 4-dimensional dataset containing lateral coordinate, m/z and drift time values, and ion signal intensity. Because ion signals of molecular phospho- and glycolipids and of silica gel and glycerol matrix-derived chemical noise occupied distinctly different regions in the m/z vs. drift time space, IMS could straightforwardly be used for spectral filtering. In this approach the region containing mainly lipid signals was marked and the dataset was reduced to values within these boundaries for all four dimensions. While the chemical noise is effectively cut out, no notable loss in the analyte signal intensities was caused. In this way, even minor compounds could be imaged with high signal contrast. The MSI images demonstrate that with the used chloroform/methanol TLC solvent system (designed for the analysis of glycolipids) also several of the main PL classes were separated. Together with the chromatographic information, improved s/n facilitated tentative identification of a large number of minor lipid components of the crude lipid extract. In summary, HPTLC-IR-MALDI MS imaging was for the first time established on a state-of-the-art MS platform providing a mass resolution of up to > 40,000. Making use of the IMS option of the instrument enabled substantial reduction of the chemical noise and greatly enhanced identification of minor components separated by HPTLC. The described workflow enables rapid lipid profiling of a complex data set.

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Posternummer: 61

Lipid-Extraktion von Chlamydomonas reinhardtii-Algenzellen für die LC-MS-Analyse

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Keywords: Chlamydomonas reinhardtii, Extraktion, Disruptionsmethode

Einleitung

Die Grünalge Chlamydomonas reinhardtii hat sich in den letzten Jahrzehnten aufgrund ihrer vielfältigen Eigenschaften als Modellorganismus für ein breites Spektrum an Forschungsfeldern etabliert. Hauptgründe sind eine gute experimentelle Zugänglichkeit, eine einfache, schnelle und kostengünstige Kultivierung, biologische Sicherheit, sowie die Vermeidung der Freisetzung von gentechnisch veränderten Organismen. In zahlreichen Experimenten ist eine Kenntnis des Lipidoms aufgrund von Veränderungen des Organismus oder der Umweltbedingungen von Interesse. Eine Extraktion der verschiedenen Lipidklassen wird durch eine starke, aus Glykoproteinen aufgebaute Zellmembran erschwert, welche mittels einer geeigneten, zu ermittelnden Disruptionsmethode zunächst geöffnet werden muss.

Experimenteller Teil

Zur Ermittlung einer geeigneten Extraktionsmethode der Lipide aus der Grünalge Chlamydomonas reinhardtii wurden zwei Bereiche betrachtet. Die aus Glykoproteinen aufgebaute, rigide Zellwand erschwert die Lipid-Extraktion. Verschiedene Disruptionsmethoden wurden zur Öffnung der Zellwand ausgewählt, ihre Durchführbarkeit überprüft sowie anschließend die mittels Lipid-Extraktion und LC-MS erhaltenen Ergebnisse im Hinblick auf die Effizienz der Öffnung und Extraktionsausbeute verglichen. Die Kugelmühle, der Ultraschrall-Zellhomogenisierer, die Mikrowelle, die Thermolyse sowie die Ausnutzung des osmotischen Druckes wurden hierzu eingesetzt. Zudem wurde die Verwendung eines geeigneten Extraktionslösungsmittels im Hinblick auf eine hohe Lipidausbeute mit einer homogen Extraktion der Lipidklassen untersucht. Hierzu wurden verschiedene ausgewählte Lösungsmittel für die Lipid-Extraktion nach Aufschluss der Zellwand mittels der Kugelmühle genutzt, die Lipide wie im ersten Bereich analysiert und die Ergebnisse verglichen.

Ergebnisse und Diskussion

Für die Ermittlung eines geeigneten Extraktionslösungsmittels wurden Zellen der Grünalge Chlamydomonas reinhardtii mittels Kugelmühlen-Disruption aufgeschlossen. Nach ersten Vorversuchen wurden die Extraktionslösungsmittel Methanol und Chloroform sowie die Gemische Chloroform/Methanol/Wasser und Methanol/Methyl-tert-butylether für die weitere Evaluation verwendet. Die Lipide aus den erhaltenen Zellextrakten wurden mit einer RP-HLPC-Methode getrennt und massenspektrometrisch detektiert. Das Extraktionslösungsmittelgemisch Methanol/Methyl-tert-butylether zeigte die größte Extraktionsausbeute. Weitere Vorteile dieses Lösungsmittelgemisches sind zum einen die geringere Dichte im Vergleich zu Chloroformgemischen, was die Abtrennung der oberen Lipid enthaltenen Phase von der wässrigen Phase und den Zellrückständen erleichtert, sowie die geringere Toxizität im Vergleich zu Chloroform.

Die Disruptionsmethoden Kugelmühle mit Stahlkugel, Thermolyse, Zellhomogenisierung mittels Ultraschall, Mikrowelle und osmotischer Druck wurden als gute Möglichkeiten zur Öffnung der Zellwand erachtet. In allen Experimenten wurde das gleiche Gewicht der erhaltenen Algensuspension eingewogen, die Extraktion mittels Methanol/Methyl-tert-butylether durchgeführt und die Lipide der verschiedenen Lipidklassen mit der RP-HPLC-MS Methode getrennt und detektiert. In Vorversuchen wurden geeignete Parameter für jede Disruptionsmethode bestimmt. Im Anschluss wurde eine Evalutation der Disruptionsmethoden mit den erarbeiteten Parametern durchgeführt.

Referenzen

Lokalisation von Doppelbindungen in Lipiden mittels photochemischer Online-Reaktion und Tandem-MS

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Keywords: Lipidanalytik, Doppelbindungsposition, Paternò-Büchi-Reaktion

Einleitung

Lipide umfassen eine Gruppe von Biomolekülen, die eine große Variation an chemischen Strukturen aufweisen und verschiedenste Aufgaben und Funktionen in unterschiedlichsten Lebensformen besitzen. Doppelbindungen haben Einfluss auf die chemischen, biochemischen und biophysikalischen Eigenschaften der Lipide. Die Lokalisation von Doppelbindungspositionen gilt als Herausforderung hinsichtlich der Komplexität der Strukturen bzw. der vorliegenden Gemische. Die photochemische Paternò-Büchi-Reaktion wurde unter Bildung von Acetonaddukten und daraus resultierender diagnostischer massenspektrometrischer Fragmentionen zur Bestimmung von Doppelbindungspositionen eingesetzt.[1] In diesem Beitrag wird diese Methode mit der HPLC-Trennung erweitert und die Anwendbarkeit auf komplexe Lipidgemische demonstriert.

Experimenteller Teil

Die Paternò-Büchi-Reaktion erfolgte im Rahmen einer Nachsäulenderivatisierung über eine Exposition von UV-Licht in einem photochemischen Reaktor unter Bereitstellung eines konstanten Acetonstroms zusätzlich zu der mobilen Phase. Zur Optimierung der Online-Reaktion wurden Parameter wie die Reaktionszeit und die konstante Flussrate des Derivatisierungsreagenzes Aceton variiert. Als Modellsubstanz wurde die einfach ungesättigte Ölsäure eingesetzt. Im Weiteren wurde die Anwendbarkeit der Methode auf ein Lipidgemisch unter Betrachtung einer Kopplung der Methode mit einer HPLC-Trennung demonstriert.

Ergebnisse und Diskussion

Mit Exposition von UV-Licht (254 nm) konnte, neben dem deprotonierten Molekül der Ölsäure, das entsprechende Acetonaddukt mit einer Massendifferenz von 58 Da detektiert werden, welches der Reaktion von Aceton mit der Doppelbindung der Fettsäure unter Ausbildung eines Oxetans zugrunde liegt. Über MS/MS-Experimente wurden diagnostische Fragmentionen gebildet, welche eine eindeutige Lokalisation der Doppelbindung ermöglichten.

Im Rahmen von betrachteten Reaktionszeiten (1,67 - 8,33 min) konnte mit zunehmender Reaktionszeit nicht auf einen höheren Umsatz der photochemischen Reaktion geschlossen werden. Vielmehr kann eine Zunahme von Nebenreaktionen angenommen werden, die auf weitere konkurrierende Radikalreaktionen mit der Ölsäure bzw. Reaktionen mit dem Acetonaddukt basieren. In Bezug auf den Acetonfluss wurden Flussraten von 0,05, 0,07 und 0,10 mL/min betrachtet, welche Volumenprozenten des Acetons zum Zeitpunkt der Reaktion von 17, 22 und 29% entsprechen. Da sowohl das Edukt als auch das Produkt mit zunehmendem Volumenprozent des Acetons abnehmen, wurde die Flussrate von 0,05 mL/min für die Reaktion gewählt.

Die Betrachtung eines Fettsäuregemisches erfolgte ausgehend von einer Kombination einer HPLC-Trennung mit der photochemischen Online-Reaktion. Die erzielte Auftrennung des Fettsäuregemisches ermöglichte eine parallele Betrachtung und Lokalisation von Doppelbindungspositionen der einzelnen Fettsäuren. Lediglich die Coelution der Positionsisomere der dreifach ungesättigten α-Linolensäure und γ-Linolensäure erschwerte eine eindeutige Zuordnung der Doppelbindungspositionen, aufgrund der geringen Intensitäten der diagnostischen Fragmentionen.

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Nutrition-dependent changes of the lipid composition of adipose tissue and liver monitored by spectroscopic methods

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Keywords: Effect of nutrition, Double bond content of lipids, MALDI MS, NMR spectroscopy, Gas chromatography

Einleitung

The intake of fat-rich food is surely one of the main reasons of obesity, but the influence of nutrition on the lipid (especially triacylglycerols (TAGs)) and fatty acyl compositions of different tissues is still not properly investigated.
In this study we combined the strengths of NMR and MS to investigate the diet impact on the lipid compositions of mice liver and different adipose depots. Compositional differences between healthy and fatty liver as well between brown, visceral and subcutaneous adipose tissues in dependence of the diets (high fat (HF) vs. standard (SD)) were first monitored by means of MALDI MS. All obtained data were also compared with established methods of lipid analysis, particularly thin-layer chromatography (TLC) and gas chromatography (GC) [1-4].

Experimenteller Teil

All solvents, free fatty acids, TAGs and the MALDI matrix (2,5-dihydroxybenzoic acid, DHB) were obtained from Sigma-Aldrich in the highest commercially available quality and used without purification. All phospholipid standards were obtained from Avanti Polar Lipids as 10 mg/mL solutions in CHCl3 and diluted to the required concentration.

All animal experiments were performed according to the German national guidelines of laboratory animal care. Tissue lipids were extracted by using methyl-tert-butyl ether (MTBE) [5]. Changes of different tissue compositions were analyzed by means of MALDI MS (Bruker Autoflex), 1H and 31P HR NMR (Bruker AVANCE-600 at 600.13 MHz for 1H and 242.88 MHz for 31P respectively) as well as HRGC (Autosys XL (Perkin Elmer) with a flame ionization detector).

Ergebnisse und Diskussion

Extracts of supplied diets, liver and three types of adipose tissues were first analyzed by MALDI MS. Qualitative compositions were additionally approved by HPTLC analysis while quantitative fatty acyl compositional analysis was performed by GC and NMR spectroscopy.

Although the diets consist almost exclusively of triacylglycerols (TAG), beside minor amounts of phosphatidylcholines in the "standard" food, the overall fatty acyl compositions of the present TAGs differ significantly: the standard diet (SD) is characterized by the presence of TAGs with longer (particularly C18), unsaturated fatty acyl residues (e.g. m/z 901.7 -TAG 54:6), whereas the high fat diet (HFD) contains nearly exclusively shorter, saturated fatty acyl residues (e.g. m/z 661.5 - TAG 36:0).

The lipid composition of diets has an extreme impact on the lipid composition of the investigated tissues. Regarding the liver, HF diet leads to incorporation of C12:0 and C14:0 and to an increased amount of TAGs (particularly 853.7–TAG 50:2; 855.7–TAG 50:1 and 881.7–TAG 52:2). GC data confirm the incorporation of shorter and saturated fatty acids. In the adipose tissues considerable differences were observed in dependence on the diet and only moderate differences in dependence on the adipose tissue type. Fatty acyl patterns of TAGs after SD conditions were inconspicuous. In contrast, the adipose tissue extracts at conditions of the HF diet, are dominated by shorter, saturated fatty acyl residues which are also predominately present in the HF diet.

The relative fatty acyl compositions of both diets and tissues of interest have also been determined by gas chromatography. Longer-chain unsaturated fatty acids (particularly linoleic acid) are nearly exclusively present in the SD, while shorter and saturated ones (particularly lauric and myristic acid) represent the majority of the fatty acids in the HF diet. Absolute quantitative data regarding the double bond content were finally obtained by high-resolution NMR spectroscopy.

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SelexIon™ Mobility Separation of Leukotriene Isomers

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Keywords: Ion mobility, lipids, diastereomers, lipidomics

Einleitung

Eicosanoids are very low abundant (IC50 pM-nM) but highly bioactive lipid mediators (LM) with highly specific structural features like double bond geometry and stereo-centers which play a crucial role in defining their bioactivity. Many eicosanoids are isoelemental and structurally closely related diastereomers and/or geometric E/Z-isomers, which make characterization by high resolution ESI-MS or even ESI-MS/MS difficult or impossible.

We developed an ion mobility method to separate isoelemental eicosanoids. SelexIon™ Differential Mobility Spectrometry (DMS) technology is capable of separating ions based on their mobility in an oscillating electrical field with asymmetric wave form. Thus, it is "orthogonal" to chromatography or mass spectrometry. We show that this method is capable of separating at least four leukotriene isomers in a sample.

Experimenteller Teil

The following synthetic standards were selected for analysis: Leukotriene B4 (LTB4), 6-trans-LTB4, 6-trans-12-epi-LTB4, 12-epi-LTB4 and 5S,12S-diHETE. LC-MS/MS measurements were conducted using an AB Sciex QTRAP® 5500 system equipped with a SelexIon™ unit coupled to an Eksigent microLC 200 HPLC system.Reverse phase chromatography was conducted using a C18, 2.6µm, 0.5 × 50mmcolumn at 15 µl/min flow rate with a binary gradient of H2O (A) and acetonitrile (B) containing 0.1% formic acid each. Gradient conditions were: 0 Min - 65% A; 0.5 - 65; 1 -30; 3 - 5; 4 - 5; 4.01 - 65; 5 – 65. The Injection volume was 4uL, column oven temperature was 50oC. Data was acquired in negative mode by SRM/MRM using the transition m/z 335->195.

Ergebnisse und Diskussion

First, we conducted an ion mobility separation of four compounds (LTB4,, 6-trans-LTB4, 6-trans-12-epi-LTB4 and 12-epi-LTB4). Using direct infusion, the minimum separation voltage (SV) required to separate those four leukotrienes was determined from the individual compounds and from a mixture of all four compounds. It was found that a minimum SV of 4100 Volts is necessary for separation with near baseline separation achievable at 4200V -4500 V.Next, the SelexIon parameters were translated to a micro-HPLC method. To this end, the compensation voltages of the compounds were mapped at a SV of 4500V.It was found that good ion mobility separation comparable to direct infusion could be achieved. A minor shift in compensation voltage compared to direct infusion was observed, which may be due to a slightly differing solvent composition of the HPLC method. Furthermore, these results highlighted the possibility of mapping the compensation voltage of isomeric compounds without the need for direct infusion, thus allowing the SelexIon-separation of endogenous compounds where no synthetic standard is available.Finally, we conducted the SelexIon-based separation of LTB4 and its diastereomer 5S, 12S-diHETE, which are extremely difficult to resolve using LC-MS/MS.LTB4 is produced by activated neutrophilic cells, whereas the formation of 5S,12S-diHETE results from a neutrophil/platelet interaction. Moreover, LTB4 is one of the strongest known chemotatctic substances while 5S,12S-diHETE is almost inactive. Hence, a differentiation of both isomers is of fundamental importance to the understanding of inflammatory processes. Using SelexIon, we show that 5S,12S-diHETE is present in residential murine peritoneal cells, while LTB4 is produced after zymosan A challenge. In conclusion, we showed that SelexIon is a powerful tool for adding an additional dimension of selectivity to chromatography for difficult separations of isomeric eicosanoids even of compounds present only at very low levels in biological samples.

Referenzen

SIMPLEX reveals a strong metabolic shift in Cav3 mutant mice

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Keywords: Lipidomics, Proteomics, Cardiomyopathy

Einleitung
"Caveolinopathies" are caused by mutations in the sarcolemmal protein Caveolin-3 and cover a group of autosomal inherited muscular disorders with a broad range of clinical severity ranging from limb-girdle-muscular to hyperCKemia dystrophy and cardiomyopathy. To gain deeper insights into the underlying molecular mechanisms, a comprehensive and representative analysis demands a deep and parallel coverage of a broad spectrum of molecular species. Therefore, we apply SIMPLEX (SImultaneous Metabolite, Protein, Lipid EXtraction procedure) [1], a novel strategy for the quantitative investigation of lipids, metabolites and proteins. Compared to unimolecular workflows, SIMPLEX offers a fundamental turn in study design, since multiple molecular classes can be accessed in parallel from one sample with equal efficiency and reproducibility.

Experimenteller Teil
The cardiac muscle of P104L mutant caveolin-3 transgenic mice [2] presenting the limb-girdle pattern and cardiomyopathy was subjected to the SIMPLEX workflow. In brief, the tissue was incubated with cold MeOH, MTBE was added and water was utilized to induce phase separation. The individual fractions containing lipids (top phase), metabolites (lower phase) and proteins (pellet) were then subjected to the individual omics workflows. Here we demonstrate (i) how SIMPLEX is suited to analyze complex tissues (e.g. heart), (ii) to investigate the interlinked proteome, lipidome and metabolome at the systems scale, and (iii) to study how a Cav3 missense mutation influences the heart metabolism of mice.

Ergebnisse und Diskussion
Application of this method in mass spectrometry based workflows allowed the simultaneous quantification of lipids, metabolites and proteins from cardiac muscle samples. The versatility of this method is shown in the transgenic Cav3 mutants, where strong metabolic shift towards a Duchenne like phenotype at the protein, lipid and metabolite level was revealed. Thereby we proved the robustness of the SIMPLEX approach to investigate interconnected systems in tissues and pointed out that SIMPLEX provides a superior strategy compared to conventional workflows and thereby allows profound insights into pathophysiological processes such as the molecular basis of cardiomyopathy.

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Posternummer: 66

Simultaneous extraction of bioactive lipids and RNA for lipidomic and transcriptomic profiling in mouse brain punches

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Organisation(en): Institute of Physiological Chemistry, University Medical Centre Mainz

Keywords: Lipidomics, Transcriptomics, Targeted LC-MS, Lipid extraction, RNA extraction

Einleitung

Lipids are molecular components which play essential roles in many physiological processes and pathological conditions, including neurodegenerative diseases. The lipids serve not only a constitutive role in the cell membrane, but also as the source for signaling molecules, such as endocannabinoids that underscore essential neurobiological functions [1],[2]. In epilepsy research lipids emerge as important candidates for biomarkers, drug targets, but also as therapeutic agents. To gain a better understanding of their specific functions and to define the altered lipid signaling networks in brain subregions that underwent epileptic seizures, we quantitatively profiled selected PLs and eCBs by LC/MRM, as well as related genes in various brain punches by the use of a dual extraction protocol for lipids and RNA [3],[4].

Experimenteller Teil

We used an advanced targeted mass spectrometry method, based on liquid chromatography (LC) and multiple reaction monitoring (MRM) to identify and quantify the lipid signals, such as endocannabinoids and their phospholipid precursors and a quantitative polymerase chain reaction (RT-qPCR) for the analysis of the corresponding RNA. The extraction of the RNA was done by using the RNeasy Mini kit from Qiagen, combined with a liquid-liquid extraction method for the isolation of lipids from the same sample.

Ergebnisse und Diskussion

Here we present the implementation of a dual extraction method of lipids and RNA from the same mouse brain punches, to investigate molecular changes in acute epilepsy versus control mice. To date, lipidomic and transcriptomic profiling is typically carried out in distinct experiments (animals/tissue regions). By combining the isolation of lipids and RNA in one experiment it is possible to not only reduce the number of animal models, but also the time and costs necessary to carry out lipidomic and transcriptomic profiling. Even more, the analysis of signaling pathways is more reliable by obtaining the molecular components from the same tissue instead from two distinct samples, circumventing thereby the pitfalls originating from tissue heterogeneity. This approach rendered a panel of lipid signatures for acute epileptic seizures encompassing phospholipids and endocannabinoids, as well as mRNAs involved in synthesis and degradation of lipid signals and neuronal activity.

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Unexpected products of the HOCl-induced oxidation of oleic acid: a study using HPTLC–ESI MS and NMR spectroscopy

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Keywords: HPTLC-ESI MS, oleic acid oxidation, hypochlorous acid

Einleitung

Reactive oxygen species (ROS) are of particular relevance in the pathogenesis of inflammatory diseases. Under inflammatory conditions, hypochlorous acid (HOCl), generated via the enzyme myeloperoxidase (MPO)[1], reacts with the double bond in the fatty acyl residues of (phospho)lipids under formation of a chlorohydrin (CH) as the main product. However, the oxidation of free fatty acids by HOCl has been investigated less detailed. Using oleic acid (OA), the simplest unsaturated fatty acid, as a model system, we investigated the product pattern of the reaction between OA and HOCl by a combination of HPTLC, different ESI MS methods and NMR spectroscopy.

Experimenteller Teil

OA, OA-13C2, decanoic acid (DA), all chemicals, solvents and primuline were from Sigma Aldrich (Taufkirchen, Germany), Merck Millipore (Darmstadt, Germany) or Roth (Karlsruhe, Germany). 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was from AVANTI Polar Lipids (Alabaster, USA).

The substances of interest were incubated separately with NaOCl (360 min, 37°C, shaking) and afterwards extracted using chloroform/methanol (1/1, v/v, +0.05% BHT)[2].

The organic layer was either directly investigated by ESI-IT MS (Bruker Daltonics, Bremen, Germany) or separated by HPTLC (silica gel 60 F254 MS-grade glass plates, Merck Millipore) using chloroform/ethanol/water/triethylamine (30/35/7/35, v/v/v/v)[3] as mobile phase. After visualizing the analytes on the HPTLC plate with the primuline, further analyses were performed with ESI-QTOF MS[2] (Bruker) after extraction using a TLC-MS interface (CAMAG, Berlin, Germany) or 13C-NMR (Bruker).

Ergebnisse und Diskussion

The reaction between POPC and HOCl results in the formation of two CH isomers as the only products (m/z 812.5, [POPC+HOCl+H]+). In contrast, the reaction of OA and HOCl does not exclusively result in the formation of CH (isomers) (m/z 333.2, [OA+HOCl-H]-) but there are also considerable amounts of dimeric (m/z 649.4, [[OA+HOCl]2-H2O-H]-) and (to a minor extent) trimeric (m/z 965.6, [[OA+HOCl]3-2H2O-H]-) products. These products could be monitored by direct infusion ESI-IT MS.

At least eight different spots could be identified by HPTLC and characterized by ESI-QTOF MS after direct elution from the HPTLC plate. Dimers and trimers were detected from different spots, i.e. with different Rf-values, while the CH was just detected as a single spot. The dimer formation can be explained by an intermolecular ether formation between the hydroxyl groups of two CH molecules, but the generation of the trimer can be exclusively explained if the carboxyl group is involved, i.e. if esters are (in addition to ethers) also generated. In order to check the possibility of ester formation, the reaction between OA and HOCl was additionally performed in the presence of DA and leads surprisingly to an ester at m/z 487.3 (negative mode) corresponding to a dimer of OA and DA. The results were confirmed by MS/MS and NMR experiments: MS/MS spectra were rather similar but showed different signal intensities for the fragment ions generated by the ester and the ether species. Finally, 13C-NMR experiments resulted in different chemical shifts for both isomeric species.

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UTILISING AN IMS-DIA-MS WORKFLOW TO CHARACTERISE AND QUANTIFY THE LIPIDOME OF A PATIENT COHORT CLINICALLY DIAGNOSED WITH OBESITY OR DIABETES

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Keywords: Lipidomics, IMS, DIA, CCS, Vion

Einleitung
Risk factors associated with increased possibility of developing diseases are commonly referred to as metabolic syndrome. Obesity is one such risk factor causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. Obesity is known to initiate inflammation, which in turn can lead to type 2 diabetes; however the exact mechanism as to how this occurs is not well understood.

Experimenteller Teil
Here, we describe a quantitative lipidomic approach, used for clinical research purposes, to reveal molecular factors that may be involved in these biomolecular processes. Human plasma samples from three biological states of varying phenotype (control, diabetic and obese) were used as case samples, with each group consisting plasma from six individuals. Samples were treated with isopropanol and centrifuged for protein precipitation. The lipid containing layer was collected and diluted to adjust the water content prior to analysis. Label-free LC-MS data were acquired in positive and negative ion mode with an IMS oa-QTof platform using a data independent analysis approach that has been described previously.

Ergebnisse und Diskussion
Unsupervised multivariate statistical analysis of the resulting data showed clear distinction between cohorts. Supervised OPLS discriminate analysis was used to filter for features of significant correlation and covariance prior to identification. Dedicated metabolomic informatics were used to process and search against LipidMaps and HMDB. Identifications matching criteria as follows, collision cross section (CCS) values <5%, mass accuracy <5ppm, ANOVA p <0.05 and fold change >2 were considered for further interrogation. Ion mobility-derived CCS measurements allowed for improved specificity with the inclusion of drift time, providing additional confidence in the identifications returned. Normalized label-free quantitation results highlighted differential expression of specific lipid classes including fatty acids, phosphatidylcholines and sphingomyelins. Additional identifications were obtained by mapping putative identifications with those from independent studies and biochemical networks.

Referenzen

Software assisted identification and quantification of oxidized phospholipids in LC-MS datasets using data-dependent and data-independent acquisition modes

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Keywords: Lipidomics, LC-MS, CID fragmentation

Einleitung

Lipid peroxidation products (LPPs) generated by oxidative stress have been recognized as biomarkers of numerous human disorders. Considering their low in vivo contents, LPPs are often quantified by targeted LC-MS/MS. However, their high structural diversity and the lack of commercial standards demand non-targeted techniques to facilitate their discovery and quantification. Such non-targeted approaches require high-resolution MS and sophisticated bioinformatics tools. Extraction, enrichment, and chromatographic separation of modified lipids have already been significantly improved, where as bioinformatics tools to identify LPPs remain the bottle-neck of high-throughput studies targeting oxidized lipids.

Experimenteller Teil

Standard glycerophospholipids (PLs) with polyunsaturated fatty acyl chains were oxidized in vitro and separated on a C18-column using aqueous methanol, acetonitrile, and isopropanol gradients (RPC). The UPLC system was coupled on-line to an ESI-IMS-QqTOF-MS (Synapt G2Si, Waters) operated in data-dependent (DDA) or data-independent acquisition (DIA, MSE) modes. LPP specific fragmentation patterns were manually annotated and used to generate spectra libraries of LPP-specific product ions. This information was combined with newly developed software tools and implemented into Progenesis QI for relative quantification of oxidized lipids. The workflow was validated using lipid extracts from rat primary cardiomyocytes cultured with or without oxidative stress inducers.

Ergebnisse und Diskussion

Lipid species are usually identified by LC-MS/MS using spectra libraries or structure-based theoretical fragmentation matches. However, most LPPs are missing in spectra libraries and currently applied fragmentation algorithms have not been optimized for oxidized lipids. Here, standard PLs with polyunsaturated fatty acyl (FA) chains were used to generate many oxidized species containing different types of modifications and analyzed by LC-MS in positive and negative ion modes. Product ions specific for truncated and oxygenated LPP species were assigned. Optimized CID conditions on the QqTOF-MS in negative ion mode provided prominent fragment ions representing sn-1 and sn-2 FA. Oxidized FA yielded specific signals indicating CID cleavage at or next to the site of oxygen addition. The optimized RPC conditions together with specific fragmentation pathways allowed, for example, to distinguish position isomers of oxidized PLs containing 9- and 13-hydroxyoctadecadienoic acyl residues. The compiled LPP fragmentation library was imported to a newly developed software capable to use the structures of possible LPP from given lipid standards for generating a library of theoretical fragment spectra. The list of LPP structures and spectra library were transferred to Progenesis QI software and validated for the LPP identification and relative quantification from lipid extracts obtained from cellular model of oxidative stress. Thus, by combining the advantages of both library search and theoretical fragmentation approaches, accurate and specific identifications of various LPP species was achieved.

Referenzen

Posternummer: 70

Applying Cook's kinetic method to the decay of doubly cationized, malonated fullerenes

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Keywords: kinetic method, malonated fullerenes, alkali metal ion affinity

Einleitung

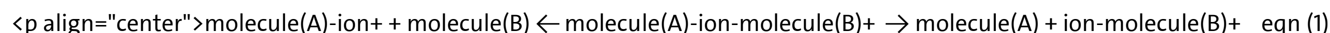
The hexakis malonate adduct to C60 with the formula C60L6 (= M) where L= C(CO2Et)2 shows an octahedral addition pattern of the six ligands. Each ligand is connected via a relatively strong covalent spiro-bridge. The compound is amenable to ESI analysis by the attachment of metal cations. Next to a set of expected ions, a doubly cationised molecule, MCat22+, can be observed. The latter ion allows two metal cations to reside on one (relatively small) neutral molecule. In dissociations, the two cations compete for one molecule, which is an unusual situation for Cook's kinetic method where normally two neutrals compete for one ion.[1,2] The study investigates the relative alkali metal cation affinities measured in CID experiments of Cat(A)MCat(B)2+ ions.

Experimenteller Teil

ESI experiments were performed with a quadrupole ion trap for the dissociation studies and a quadrupole time-of-flight hybrid with higher resolution was used to confirm the assignment of ions. Hexakis and pentakis malonate adducts to C-60 (o-DCB or DCM:MeOH 1:1 (v:v)) were respectively mixed with two alkali metal salt solutions (MeOH) and further diluted with ACN resulting in concentrations of 10-5 mol·L-1 for both the malonated C60 and the two alkali metal cations. Salts with the alkali metal cations from Li+ to Cs+ were used while studying always neighbouring metals.

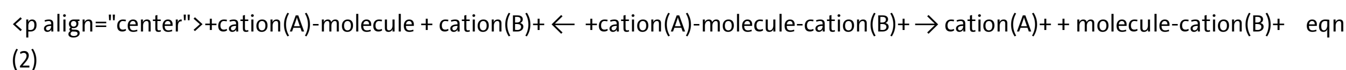
Ergebnisse und Diskussion

The usual application of Cook's kinetic method is concerned with a situation in which two neutral molecules compete for one ion. As a result the relative ion affinities of those molecules can be derived in dissociation experiments evaluating the relative abundances of the daughter ions. Equation 1 may serve as a general example of this:



<p align="center">molecule(A)-ion+ + molecule(B) ← molecule(A)-ion-molecule(B)+ → molecule(A) + ion-molecule(B)+   eqn (1)

If the ion is for instance a metal cation, the exchange to a different metal is commonly easily achieved. While this allows to evaluate the same competition for different metals, it remains frustrating that this experiment cannot reveal if the new metal ion is stronger bound to one of the molecules or not, that is the relative affinity of both metal cations to the molecule remains unknown. The observed MCat22+ ion, however, allows exactly to obtain this information, as in this case the two cations compete for the same neutral molecule. Equation 2 illustrates the situation:



<p align="center">+cation(A)-molecule + cation(B)+ ← +cation(A)-molecule-cation(B)+ → cation(A)+ + molecule-cation(B)+   eqn (2)

In dissociations the smaller cation stays preferably with the molecule, when cation(A) and cation(B) are neighbouring alkali metals. Thus, the relative metal cation affinities follow the order Li+ > Na+ > K+ > Rb+ > Cs+. The relative affinities show more pronounced differences for the smaller metal ions. This affinity order follows roughly the charge density of the ions allowing for stronger interaction when the ion is smaller. It is well-established that the alkali metal cations bind to the ligands (oxygen), rather than to the fullerene. The pentakis adduct shows a similar trend. MCat22+ ions of lower ligand attainment could not be generated so far due to the Coulomb repulsion exceeding the stabilization of the two charges. Current work extends to other mono-valent metal ions.

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Fragmentation of the diethylamino-sidegroup: A fundamental concept

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Keywords: Fragmentation, Photodissoziation, Mechanism

Einleitung

Diethylamino-sidechains are part of several organic and biological compounds which are investigated with high resolution mass spectrometry. The fragmentation of the diethylamino-sidegroup has been investigated in case of the dye Rhodamine B [1] and two basic mechanisms were proposed for the main loss of C3H8. In order to receive further information about the undergoing mechanism a model dye system was synthesized. The energy dependent fragmentation with short laser pulses at 532 nm as well as quantum chemistry calculations and comparison with other investigated dyes allow us to express a rule for the observation of characteristic fragments and the undergoing mechanism.

Experimenteller Teil

All PD mass spectra have been recorded on a Bruker Apex FT-ICR equipped with an Apollo III electrospray ionization (ESI) source with a superconducting 7.0 T magnet (Bruker Daltoniks, Bremen, Germany). As a light source for the VisPD fragmentation a Nd:YAG Laser (Continuum) has been used with different pulse length. The instrument was mass calibrated on arginine clusters to guarantee a resolution error under 2 ppm. The molecular peak was isolated in the ICR cell to get isotopic free spectra. In addition to experimental measurements DFT calculations were performed using Gaussian. All geometric optimization were made using B3LYB with a 6-311+(2df)(2pd) basic set, transition states were detected via QST3 and verified with intrinsic reaction coordinate (IRC) and frequency calculations.

Ergebnisse und Diskussion

The investigated model system is a pair of two molecules, one without a positive charge and one with a fixed charge over the whole molecule. Both molecules show fragments at Photodissoziation with 532 nm, whereat the fragmentation pattern changes drastically. In case of the fixed charge the most intense fragment is the loss of C3H8 in combination with a loss of C2H6. If the charge is provided by an additional proton the loss of C3H8 is still dominating the fragmentation pattern, but several small fragments, e.g. the loss of C2H5 are observed.

The change in the fragmentation pattern is caused by a geometry change of the sidegroup due to charge position effects. This was proved by comparison with several other dye systems which were partly modified for the investigation of this effect and DFT-calculations.

Beside the effect of the charge position the undergoing mechanism was investigated. Here the observed fragments indicate a two-step radical mechanism in case of the C3H8-loss. DFT-calculations support the radical mechanism as well; the radical loss is energetically more favorable than the concerted mechanism.

With the investigated set of dye molecules and the information from energy dependent measurements as well as DFT-calculations it is now possible to understand the occurred mechanisms and to predict the observed fragments of the diethylamino-sidegroup.

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Spin-isomers in the gas-phase: reactivity of ferracyclobutadienes

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Organisation(en): Universität Bonn, Deutschland

Keywords: gas phase reactivity, alkyne metathesis, spin isomers, iron

Einleitung

Metallacyclobutadienes are often discussed as intermediates in alkyne metathesis reactions although only a handful of this highly reactive species could be isolated and characterized so far. As a new class of ferracyclobutadiyenes [FeL3(C3R3)]+ (L = CO, PMe3; R = NMe2) has been synthesized in the group of Prof. Filipou from the University of Bonn, the gas-phase reactivity of these highly unusual species is presented and discussed here.

Experimenteller Teil

The experiments have been performed with a Bruker Apex IV FT-ICR equipped with 7 T magnet, Apollo ESI-source, and an enhanced gas inlet system including two pulse- and two leak valves. Pulses of argon 5.0 are used for CID and thermalization. IRMPD is done with a CO2-Laser with max. 25 W. Propyne, 1-butyne and 2-butyne were chosen as reaction gases. A typical experiment starts with the accumulation of the ferracyclobutadienes in the hexapole of the instrument, fragmentation via IRMPD, mass selection of the desired coordinatively unsaturated ferracyclobutadiene, thermalisation, re-isolation, variation of the reaction time in presence of the alkyne, excitation and detection. 16-500 scans were accumulated. Reaction products were identified by accurate mass and by additional mass selection/fragmentation experiments.

Ergebnisse und Diskussion

ESI is a convenient ionization method for the cationic ferracyclobutadiene complexes. The fragmentation cascades induced by CID or IRMPD start with the successive loss of monodentate ligands until the "naked" 10-VE-ferracyclobutadiene core is reached. Thus, a full series of 16 – 10 VE ferracyclobutadienes are experimentally accessible. The mass selected coordinatively unsaturated ferracyclobutadienes show interesting reactivity towards small alkynes. The 12 VE-species in particular display complex non-monoexponential kinetics even after intensive thermalization processes indicating the presence of isomeric species with strongly differing reactivity. Quantum chemical calculations confirm the presence of extremely small singlet-triplet gaps as well as the observed reactivity patterns.

Referenzen

Investigation of the electronic properties of polyynes and polyyne rotaxanes by silver induced gas-phase reactivity

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Keywords: Polyyne, Rotaxane, Silver, Density Functional Theory Calculations

Einleitung

Polyynes are carbon-rich compounds representing the sp-hybridized carbon allotrope carbyne. They show unusual electronic and optical properties and are therefore under investigation for the application in molecular wires, non-linear optical materials or nano-electronic devices. The inherent high reactivity of the conjugated triple-bonds can be controlled by applying bulky protecting groups to the ends of the carbon chain [1]. Polyynes that are additionally shielded by a non-covalently bound macrocycle are called rotaxanes [2]. The present investigation focusses on the gas-phase reaction behavior of Ag+-adducts of "free" polyynes and their rotaxane counterparts. The Ag+-adducts are formed by ESI and studied in CID experiments. Special emphasis is on the reactivity induced by the Ag+-ion and the different behavior of both types of molecules.

Experimenteller Teil

The experiments are performed by electrospray ionization and accompanied by density functional calculations using turbomole 6.6 with the PBE functional and def2-TZVP basis set and Grimme D3 dispersion correction.

Ergebnisse und Diskussion

Following an earlier study into the cross linking of polyynes by laser desorption mass spectrometry [3], the present investigation focusses on the gas-phase reaction behavior of polyyne / Ag+ adducts. Polyynes of different chain length and terminated by the supertrityl group are compared with their corresponding rotaxanes. With polyynes, the silver cation attaches preferably to the outermost triple bonds, simultaneously coordinating to the aryl ring of the protecting supertrityl group. Upon collisional activation, the silver inserts into the carbon chain, predominantly at the end and less frequently towards the middle of the chain. The insertion is followed by dissociation into a neutral silver acetylide and a positively charged supertrityl group that may still contain up to two acetylenic units from the former polyyne chain. In the case of the rotaxanes, the preferred coordination site of the silver is the phenanthroline part of the macrocycle. Upon activation, the silver still inserts into the polyyne chain, but the macrocycle has a strong influence on the position of the insertion. DFT calculations confirm the observed experimental findings and help compose the mechanistical picture of the reaction behaviour.

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Gas-Phase Experiments with Polyynes: Laser Activation and Ag+-Induced Reactivity

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Keywords: Oligoynes; Gas Phase; Aggregation; Ions; MALDI; Electrospray.

Einleitung
End group-protected linear polyynes of the composition Tr*–(C≡C)n–Tr* with Tr* representing the super trityl group (Tr* = (tBu2(C6H3))3C, n = 2, 4, 6, 8, 10) and tBu–(C≡C)6–tBu with tBu being the tertiary butyl group have been studied by laser desorption ionisation (LDI) time-of-flight (TOF) mass spectrometry.

Experimenteller Teil
ESI-QIT

(MA)LDI-TOF

Ergebnisse und Diskussion
tBu terminated polyyne molecules show considerably higher stability during laser activation than Tr*-end-capped polyynes. A key feature is the abundant formation of oligomeric species upon laser activation. Tandem mass spectrometry reveals strong bonding within the oligomers which indicates cross-linking of the former polyynes. The process is more abundantly occurring and less energy demanding than the laser-induced coalescence of C60. Cross-linking is more efficient with the smaller end group (tBu) and larger oligomers are formed when the chain length of the polyyne increases, both a result of enhanced interaction of the triple bonds in neighbouring chains. The presence of the matrix molecules in matrix-assisted (MA)LDI hinders the polyyne interaction and oligomer formation is markedly reduced.[1] Ag+ attachment makes the molecules accessible to electrospray ionisation (ESI). Electrospraying the polyynes from solutions containing AgTFA results in the formation of [M+Ag]+ and [M+2Ag]2+ at considerably high intensity and in the formation of the dimeric species [2M+Ag]+ (with M being the neutral polyyne molecule) for most of the polyynes under investigation. For both monomeric species, collisional activation leads to Ag+-insertion into the sp-carbon chain resulting in the formation of a carbenium ion ([Tr*(C2)x]+) and a silver acetylide (Tr*(C2)yAg and [Ag+Tr*(C2)yAg]+, respectively). Collision-induced dissociation (CID) of the Ag+-bridged dimer reveals that there is only one notable fragmentation channel: [2M+Ag]+ → [M+Ag]+ + M. Since the formation of hetero dimers (different chain lengths) is observed for mixed compound solutions, the relative silver-ion affinities of the polyynes were investigated applying the kinetic method.

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Characterization of UV transformation products of sulfamethoxazole

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Organisation(en): Universität Münster, Deutschland

Keywords: sulfamethoxazole, transformation products, HPLC-ESI-MS, UV degradation, photolysis

Einleitung

The removal of micropollutants from surface waters is a topic of growing awareness. Not all xenobiotics are sufficiently removed by municipal wastewater treatment plants. As a result, additional disinfection steps such as ozonation, chlorination or direct photolysis utilizing UV light are investigated. Many of these procedures are already well elucidated regarding degradation efficiency or energy costs but only few studies deal with the identification of formed transformation products and their toxicity.

In the present study, the photolytic degradation of the frequently used antibiotic sulfamethoxazole (SMX) using polychromatic UV light is investigated focusing on the identification and characterization of formed transformation products via high performance liquid chromatography coupled to electrospray ionization mass spectrometry (HPLC-ESI-MS).

Experimenteller Teil

The oxidative degradation of SMX using direct photolysis with UV light has been realized with a high-pressure mercury lamp (250 W) emitting light in the range of 220-500 nm. A shutter allowed for a precise control of the irradiation time enabling the determination of an irradiation time dependent characterization of transformation products. Additionally, the SMX solutions were adjusted to pH 3 and pH 8 using a phosphate buffer to study the pH dependency of product generation.

For the characterization of formed transformation products, high resolution time-of-flight mass spectrometry and fragmentation experiments (MSn) using ion trap mass spectrometry, both hyphenated to high performance liquid chromatography, were used.

Ergebnisse und Diskussion

The photolytic degradation of the antibiotic sulfamethoxazole in aqueous solutions has been investigated to characterize transformation products. Plotting the HPLC-ESI-MS chromatograms against the irradiation time results in a 3D-plot, which allows for a broad overview on the generated transformation products. Oxidizing SMX leads to a large variety of products. Some of these, as mono- or dihydroxylated SMX, have already been published, whereas some are newly found. In total, more than 20 different mass-to-charge ratios could be found, ten of which have been published before.

It was shown that the pH value has a great impact on how SMX is degraded and what transformation products are formed. Most obvious is the greatly reduced degradation time of SMX at pH 3. To degrade SMX to a similar level, the photolysis needs 18 min at pH 8 whereas it takes only 4 min at pH 3. Additionally, the main transformation product varies depending on the used pH value. While the photolysis of SMX at pH 3 mainly leads to an isomerization of SMX itself, at pH 8, a broader variety of main products is formed. There is also a difference in the color of the irradiated solutions. While irradiation at pH 3 leads to a yellow solution, at pH 8, small black particles are formed additionally, resulting in a much darker, yellow-grey suspension.

Referenzen

Top-down Approach for Electrochemical Metabolite Synthesis

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Organisation(en): Universität Münster, Deutschland

Keywords: Electrochemistry, Mass Spectrometry, Liquid Chromatography, Metabolite Synthesis

Einleitung
The elucidation of the metabolism of drug candidates is of great importance during drug development for toxicity assessment. Conventionally, biological in vivo and in vitro assays are applied for metabolism studies. The major disadvantage of these methods is the use of animals and the difficult identification of reactive metabolites. Electrochemistry (EC) coupled to mass spectrometry (MS) has been proven to serve as an alternative purely instrumental approach for simulating oxidative metabolism reactions. EC/MS experiments are mostly performed using low volume electrochemical cells resulting only in small amounts of the metabolites. Since larger quantities are needed as reference standards and for toxicity testing, a top-down approach for the electrochemical synthesis of drug metabolites was developed using MS as monitoring technique.

Experimenteller Teil
A preparative electrochemical bulk cell was developed and optimized. Therefore, paracetamol was investigated as a model compound. The cell potential was controlled using a home-made seven-channel potentiostat. The setup was first screened for the potential of the highest conversion rate. Therefore, a mass voltammogram was recorded by applying different constant potentials to the working electrode. The oxidized solutions were analyzed by means of atmospheric pressure chemical ionization (APCI)-MS. Afterwards, reaction conditions influencing the oxidation behavior were studied and optimized using LC/ESI-MS. These include the composition of the solvent, the influence of the corresponding electrolyte concentration and the substrate concentration.

Ergebnisse und Diskussion
The geometry of the implemented electrochemical cell is based on a three electrode setup in a 200 mL beaker. The working electrode (WE) is made from a platinum mesh and the counter electrode (CE) is a coiled platinum wire. The potential was applied against an Ag/AgCl reference electrode placed between WE and CE. A home-built seven channel potentiostat was used for potential control enabling the simultaneous operation of seven cells. In order to develop a method for optimizing the electrochemical oxidation of paracetamol within this cell, first, the potential of the highest conversion rate resulting in the electrophilic metabolite of paracetamol, N-acetyl-p-benzoquinone imine (NAPQI), was determined. Therefore, a solution of paracetamol was oxidized at constant potentials in steps of 200 mV. The oxidized solutions were analyzed by means of APCI-MS in order to detect low-polarity quinoid metabolites of paracetamol. The determined optimum potential was then applied for further investigations concerning the optimization of the reaction conditions. These include the composition of the solvent used for the oxidation as well as the influence of the concentration of the applied electrolyte. In both experiments, the oxidation of paracetamol was monitored over 2 h analyzing aliquots taken throughout the whole experiment of the oxidized solution by means of LC/ESI-MS. Furthermore, the influence of the substrate concentration for the electrochemical conversion was investigated.

Referenzen

Posternummer: 77

ASAP®-MS as a fast and reliable alternative for the detection of indigoid colorants in historic textile finds

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Keywords: ASAP®-MS, dyestuff analysis, prehistoric textiles

Einleitung

Archaeological textile finds are testimonies of one of the earliest human craft technologies. The scientific investigation of these objects and the characterisation of the used materials, e.g. the fibers and dyestuffs, are an access to knowledge about long-gone, ancient societies. However, organic materials like textiles decompose rapidly in the archeological context and usually samples are available only in very limited amounts. Therefore, non-destructive or minimally invasive techniques are preferred. In this study ASAP®-MS is applied to analyse indigoid colorants in tiny fragments of historic textiles without any preparation of the sample prior to their measurement.

Experimenteller Teil

The minimally invasive investigations were performed using ASAP®-MS (atmospheric solids analysis probe). This ambient mass spectrometry technique allows a rapid and direct sampling; hereby a couple of fibers are sufficient for the detection of indigoid-like dyes. In addition to ASAP®-MS and to confirm its results, ATR-FTIR, Raman, HPLC-DAD and LC-MS/MS experiments were performed.

The investigated blueish textiles date back to several different ancient Peruvian cultures, and they differ in age, type of fiber (animal- and plant-derived fibers) and their state of preservation.

Ergebnisse und Diskussion

Across all cultures and over all time periods, indigoid colorants have been used to dye textiles [1]. Thereby the vat dye indigo was one of the most important organic dyestuffs for creating blue hues in the ancient world. For the identification of this colorant different invasive and non-destructive approaches like Raman or IR spectroscopy exist. However, the use of classical Raman and IR spectroscopy without any prior preparation or treatment seems troublesome because the dye is always a minor component in these heterogeneous systems. Thus, sometimes the colorant's signals are superimposed by signals originating from the fiber matrix or from organic and/or inorganic impurity attachments, contaminants and/or degradation products from the fiber. In addition, the occurrence of fluorescence is a problem in some of the Raman experiments. The ASAP®-MS technique is an alternative method.

In the ASAP® experiments for all blue-colored samples the presence of indigoid-like dyes was detected - regardless of the fiber material, the age and the state of preservation. Thereby determinations were completed in less than 1 min [2].

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Posternummer: 78

Arsenic Speciation of Roxarsone and its Electrochemically Generated Transformation Products

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Keywords: Speciation, Arsenic, Electrochemistry, Mass Spectrometry, Hydrophilic Interaction Liquid Chromatography

Einleitung

Roxarsone is an arsenic containing animal feed additive which is presumed to be excreted unmetabolized from the organism. However, the detailed metabolic pathway is not entirely known and for this reason, toxic effects cannot be excluded. Therefore, the oxidative transformation of roxarsone was investigated using a purely instrumental approach consisting of an electrochemical cell coupled online to an electrospray ionization mass spectrometer (ESI-MS). This setup has been proven to be a valuable tool in metabolism simulation. Hyphenation with liquid chromatography (LC) and inductively coupled plasma mass spectrometry (ICP-MS) as well as ESI-MS further enables quantification and characterization of the obtained metabolites.

Experimenteller Teil

The oxidative transformation of roxarsone was investigated using EC/ESI-MS. By potential ramp application to the working electrode of a flow-through electrochemical cell, a mass voltammogram was recorded enabling an easy identification of the oxidation products. Next, the products were separated using hydrophilic interaction liquid chromatography (HILIC) coupled to ICP-MS as well as ESI-MS. The application of both, elemental and molecular MS, enabled quantification and species identification. Further, adduct formation of the electrogenerated products was performed by adding a solution of glutathione and human serum albumin, respectively, to the effluent of the cell. Afterwards, (LC/)ESI-MS analysis was carried out.

Ergebnisse und Diskussion

The oxidative transformation of the arsenic containing drug roxarsone was investigated using EC/ESI-MS in order to elucidate potential metabolites of the drug. First, a mass voltammogram was recorded, where mass spectra are plotted against the applied potential. By this means, oxidation products of roxarsone were identified including the toxic inorganic species arsenate (AsV) as well as the mono-, di- and trihydroxylated and the corresponding dehydrogenated species. In order to characterize the obtained products regarding their polarity and isomer formation, a separation was carried out by means of HILIC coupled to ICP-MS and ESI-MS. The complementary application of elemental and molecular MS provided quantification and species identification. For the first time, not only potential arsenic metabolites could be identified, but also non-arsenic ones resulting from a carbon-arsenic bond cleavage. Furthermore, the reactivity of the transformation products towards biomolecules was analyzed by means of adduct formation with biomolecules. First, adduct formation with the tripeptide glutathione was carried out resulting in two different adducts with electrophilic quinoide oxidation products. One of these is an arsenic containing product, the other doesn’t contain arsenic. The same adducts were also found for the adduct formation with the more complex protein human serum albumin. Thus, not only arsenate is a potential health risk resulting from the oxidative transformation of roxarsone during metabolism or post-excretional degradation, but also quinones.

Referenzen

Absolute quantification of a human blood plasma biomarker for sepsis by MALDI-TOF mass spectrometry

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Keywords: Blood plasma, sepsis biomarker, solid-phase extraction, MALDI-TOF mass spectrometry, absolute quantification

Einleitung

Sepsis, the infectious form of the systemic inflammatory response syndrome (SIRS), is a severe disease with increasing incidence and high mortality. Treatment of sepsis is most effective in its early stages, but diagnosis is difficult at this time [1]. Currently, no reliable biomarkers are available for the diagnosis of sepsis. Hence, the development of new biomarkers that allow early diagnosis as well as differentiation from non-infectious SIRS is of tremendous importance. A proteolytic fragment (referred to as CAAT4.8) of the C-terminal section of the acute-phase protein Alpha-1-Antitrypsin is considered to be a promising biomarker, but validation is necessary [2].

We present here the prove-of-principle of a fast and cost-effective MALDI-TOF-based assay for the absolute quantification of a large peptide biomarker for sepsis in human plasma.

Experimenteller Teil

Human plasma samples (supplied by Integrated Biobank Jena, IBBJ) were spiked with definite amounts of stable isotope labelled synthetic CAAT4.8. Subsequently, spiked samples were purified by solid-phase extraction (SPE) and the eluates were applied onto a MALDI steel target. Data acquisition was performed automatically on a MALDI-TOF/TOF mass spectrometer (Autoflex Speed, Bruker Daltonics) using reflector positive mode. Absolute quantification was done by determining signal area ratios of the peaks for endogenous CAAT4.8 and its stable isotope labelled synthetic counterpart.

Ergebnisse und Diskussion

Synthetic CAAT4.8 and human plasma samples were used to develop a purification procedure and to examine the suitability of MALDI-TOF mass spectrometry (MS) for absolute quantification of a large peptide.

In spectra acquired by means of MALDI MS, no linear correlation between analyte concentration and signal response is observed [3]. Hence, the first step of the assay development was to determine a calibration function that allows the calculation of concentration values out of signal area ratios. In the following step, different SPE materials were checked for their ability to retain the analyte and finally, a C18 and a mixed-mode (cation exchange) material were selected. A concentration of 10 µg/ml of isotope labelled CAAT4.8 in plasma was found to be sufficient for reliable detection. This should enable the quantification of endogenous CAAT4.8 even in plasma samples of healthy persons. However, the analysis of plasma from a sepsis patient yielded a concentration of ≤10 µg/ml for CAAT4.8, which is lower than the expected concentration. In a subsequent experiment, the stability of the analyte was examined by incubation of the synthetic internal standard with neat blood plasma for different time intervals in the cold room. Quantification of CAAT4.8 in these samples revealed a progressive concentration reduction over time, which indicates that degradation and/or adsorption processes are occurring.

The use of SPE well-plates for plasma purification and MALDI-TOF MS for sample analysis enabled the cost-effective determination of the CAAT4.8 concentration within only a few hours, which makes the assay an ideal tool for large-scale studies of peptide biomarkers.

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Characterization of molecular changes in bone cells during osteogenesis

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Keywords: bone cells, lipids, proteins, label-free quantification, expression levels

Einleitung

During fracture healing, mesenchymal stem cells (MSC) differentiate to osteoblasts to mineralize the soft callus leading to bone consolidation. However, fibroblasts located abundantly in the site of injury proliferate and initiate mineralization by responding to the same signals as osteoblasts. In large defect mineralized fibrocartiliganous callus leads to pseudarthrosis. The study hypothesized that minute cellular differences between osteoblast-like fibroblasts and MSCs-derived osteoblasts result in discrepant tissue properties during bone healing. Therefore, we characterized the lipidome and proteome of fibroblasts and MSC before and after osteogenic differentiation in vitro using several mass spectrometric methods in order to evaluate this hypothesis.

Experimenteller Teil

MSCs and fibroblasts obtained from reaming-debris underwent osteogenic differentiation in vitro. After 4 days cells were washed with PBS and stored at 80°C. For proteomic analysis cells were homogenized, enzymatically digested, peptides were purified and analyzed using a nanoHPLC -MS/MS based label free quantitative approach (MaxQuant [1]). For lipidomics analysis cell homogenates were spotted onto the sample holder, covered with matrix and measured using MALDI MS imaging. All experiments were performed on an orbital trapping mass spectrometer (Q Exactive, Thermo Fisher Scientific). For each cell type three biological and three technical replicates were analyzed. Statistical data analysis was performed using Perseus [1].

Ergebnisse und Diskussion

Proteomics: In total more than 2200 proteins were found in the four cells types, several of which being differentially expressed with statistical significance. We found that the protein expressions in stem cells and fibroblast cells were different before and also after the osteogenesis, pointing out that their behavior in bone repair might be different. Analysis of the biological relevance of the differentially expressed proteins is still ongoing.

Lipidomics: We found more than 100 signals in the MALDI MS spectra which were differentially expressed with statistical significance in the four cell types and could be assigned to lipids by database search (Metlin[2]). The label-free quantitative approach showed that expression levels of these lipids were different in stem cells and fibroblasts, but after the osteogenic differentiation both osteoblast types showed very similar expression level. Verification of the assigned lipid species via MS/MS is still ongoing.

Conclusion: Mesenchymal stem cells and fibroblast cells both have a specific protein and lipid profile. After the osteogenesis the protein expression levels in osteoblasts derived from MSCs and fibroblasts still show differences, but lipid expression levels are very similar.

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Comparative Studies on the Characterization of Bacteria Cultures by MALDI-MS in Linear and Reflectron-TOF modes and by Using Proteolytic Digestion

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Keywords: Biotyping, MALDI-TOF-MS, reflectron mode, tryptic digest

Einleitung
Rapid identification of microorganisms by MALDI-Biotyping is increasingly used in the clinical laboratory [1-4]. In this highly standardized technique linear TOF mass spectrometers at low mass resolution are used to characterize microorganisms on the basis of varying ion profiles (corresponding mainly to ribosomal proteins) in a typical m/z range of 2,000 to 20,000. However, a differentiation of bacteria on a subspecies level is not generally possible. In the present study we compared ion profiles that were obtained from bacteria cultures in linear and reflectron mode of a research grade TOF mass spectrometer. In order to improve the information content of the mass spectra obtained, first attempts to analyze proteolytic digestions of crude bacteria extracts were performed.

Experimenteller Teil
11 different bacterial strains from 3 genera (Escherichia, Pseudomonas and Staphylococcus) were cultured. Peptides and proteins were extracted according to modified standard protocols [5]. Part of the samples were digested using trypsin. α-cyano-4-hydroxycinnamic acid was used as MALDI matrix. For mass spectrometric analysis we used a 4800 MALDI TOF/TOF™ Analyzer (ABSciex) in linear and reflectron mode. For comparison a microflex LT MALDI Biotyper (Bruker) was employed. While the higher mass range up to 20,000 Da was covered well in the linear mode at a mass resolution of about 600, the reflectron mode of the TOF/TOF analyzer enabled the analysis of genuine and proteolytic peptides with a resolution of ~10,000. RapidMiner software was used for principal component analysis (PCA).

Ergebnisse und Diskussion
Generally similar mass spectral profiles were obtained with the microflex LT MALDI Biotyper and in the linear TOF mode of the 4800 mass spectrometer. In reflectron mode the expected improvement in accuracy in the low m/z range (700-4000 Da) was achieved. However, a sizable reduction in the ion signal intensities was observed in comparison to linear TOF data in particular in the high mass range (4000-7000 Da). Interestingly, the intensity loss was more or less pronounced depending on the bacterial species under inspection. Next to possible ion transmission issues (i.e., a generally lower overall sensitivity in the reflectron mode), protein species-depending extents of metastable ion fragmentation occurred. This assumption was corroborated by the detection of the neutral fragments by the linear TOF detector upon use of a deflecting electrical field. To circumvent this problem, tryptic digestions of extracted native and reduced and alkylated proteins were analyzed. First results suggesting that this approach may lead to an improved differentiation of bacteria will be presented.

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Verification of sulfur mustard exposure using µLC-ESI HR MS/MS after direct plasma proteolysis

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Keywords: Albumin-adducts, high resolution mass spectrometry, hydroxyethylthioethyl, verification analysis, vesicant

Einleitung

Sulfur mustard (SM) is a banned chemical warfare agent that belongs to the class of vesicants. SM may lead to painful erythema, blistering, temporary blindness, and respiratory problems [1].

After incorporation, SM rapidly alkylates diverse endogenous bio-macromolecules like DNA and proteins (adducts) and forms the characteristic hydroxyethylthioethyl (HETE) moiety [2]. After proteolysis, these covalent adducts are well suited for verification analysis using modern mass spectrometric techniques [2].

A method to detect the characteristic SM-adduct of human serum albumin (HSA) alkylated at its Cys34 residue is presented. In order to avoid an expensive and time-consuming HSA extraction step, a procedure for direct proteolysis of human plasma was developed [3].

Experimenteller Teil

Human plasma exposed to SM was enzymatically degraded with pronase avoiding any initial preparation step. The resulting alkylated dipeptide HETE-cysteine-proline (HETE-CP) was detected by micro-liquid chromatography electrospray-ionization high-resolution tandem-mass spectrometry (µLC-ESI HR MS/MS). In order to optimize the proteolysis conditions in terms of reproducibility and yield, kinetics were investigated. Furthermore, two different mass spectrometers – a triple quadrupole mass spectrometer (4000 QTrap) and a hybrid quadrupole time-of-flight (TOF) MS system (TT5600+) – were compared with respect to selectivity and sensitivity.

Ergebnisse und Diskussion

HETE-CP eluted as a narrow peak with a retention time of 8.5 min when using a chromatographic temperature of 60°C.

The TT5600+-system allowed detection of fragment ions with a mass accuracy always better than 14 ppm (better than 3 ppm for half of the detected ions).

Proteolysis kinetics of SM-treated human serum, citrate-, and heparin-plasma revealed that a pronase concentration of 3 mg/mL at 37°C lead to a stable plateau of HETE-CP concentration after 90 min of incubation. In contrast, EDTA-plasma showed a reduced HETE-CP formation velocity due to enzyme inhibition by the Ca2+-chelating effect of the anticoagulant EDTA. However, when using pronase concentrations of 12 mg/mL EDTA-plasma samples from different lots also reached a stable HETE-CP plateau within 120 min.

The limit of detection based on the peak area ratio of the two most intense transitions was determined to be at 156 nM SM in plasma (4000 QTrap) and 19.5 nM SM (TT5600+). This effect was due to the high resolution of the TOF analyzer of the TT5600+ which detects fragments with their exact mass. Therefore, the TT5600+ reduces the impact of any interference and is predestinated for such complex matrices.

The method was successfully applied to in vitro and in vivo samples of real human cases of SM-poisoning. Furthermore, measurements of patient samples taken at different time points suggest a period of up to 40 days after exposure for successful verification according to the turnover of the natural protein.

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Posternummer: 83

A new ICP-TOFMS for the analysis of micro- and nanosamples

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Organisation(en): TOFWERK, Schweiz

Keywords: ICP-MS, nanoparticles, laser ablation

Einleitung
A growing interest in the elemental analysis of very small samples such as microdroplets, nanoparticles or single cells has created a high demand for faster and more sensitive inductively coupled plasma mass spectrometers (ICP-MS). Simultaneous detection over the entire mass range and high temporal resolution are key parameters for the accurate and precise measurement of very short transient signals (200 µs- 10 ms) produced from small sample volumes. This level of performance cannot be achieved by quadrupole-and sector-field-based systems but requires a time-of-flight mass spectrometer.

Experimenteller Teil
All ICP-MS measurements were carried out on a TOFWERK icpTOF in combination with either the standard liquid introduction system or the laser ablation system equipped with a fast washout Tube-cell from ETH Zürich. For the measurement of nanoparticles hydrogen was used in the Q-cell to remove ArO interferences and improve 56Fe detection. Nanoparticles were diluted with milliQ water to the final concentration of 105 particles/ml and sonicated for 10 min before every dilution step. Lake water was dialyzed and diluted by a factor of 100 in milliQ water prior to the analysis.

Ergebnisse und Diskussion
We present the icpTOF, a new ICP-MS featuring a time-of-flight mass analyser. It records the entire mass spectrum with µs time resolution. The analytical performance of the new instrument will be demonstrated on several selected applications, covering the following topics:

1) Detection and quantitative determination of elemental composition and size of engineered nanoparticles present in aqueous media at very low amounts (103-105 particles/ml).

2) Multi-element analysis of geological liquid micro-samples by laser ablation. Strengths and features of fast simultaneous detection will be discussed and demonstrated in several applied studies.

Referenzen

Speciation analysis to investigate the interaction between implant metals and blood proteins by means of LC-ESI-MS and LC-ICP-MS

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Keywords: ICP-MS, speciation analysis, cobalt

Einleitung

The implantation of hip joint endoprosthesis has developed to one of the most important procedures in the field of medical surgery. Studies revealed that these implants sometimes cause a significant release of its alloy metals associated with abrasion effects due to friction and corrosion resulting in high concentrations in blood and urine. In this work, the release of cobalt and its interactions with blood proteins was investigated. Simulated and real samples from an implant patient were analyzed by means of high-performance liquid chromatography (HPLC) in combination with inductively coupled plasma-mass spectrometry (ICP-MS) and electrospray ionization-high resolution mass spectrometry (ESI-HRMS).

Experimenteller Teil

The hyphenation of HPLC with ICP-MS and ESI-HRMS has developed to an important tool in speciation analysis accessing quantitation data next to the molecular information. First, the bioavailability and the release of the respective alloy metal cobalt has been studied by means of ICP-MS revealing high amounts of cobalt in a blood sample of a patient with CoCrMo-hip implant. To access species information, HPLC was coupled to ICP-MS in order to separate present cobalt complexes with human serum albumin (HSA) and hemoglobin in simulated as well as real samples. Subsequently, the detected species were further investigated using HPLC-ESI-HRMS. By means of a high mass resolution with a high mass accuracy, the found signals were assigned to specific metal-protein adducts.

Ergebnisse und Diskussion

First, a method for the determination of cobalt in biological matrices was developed by means of standard addition and evaluated using a reference material (ClinChek, Level II).Subsequently, the developed method was used for the quantitation of Co in a blood sample of a healthy volunteer and in a blood sample of a patient with CoCrMo-hip implant. The Co concentration of the volunteer sample was in accordance to the expectations in the low µg·L-1range (0.33 µg·L-1), whereas the patient sample showed a high abundance of Co (700 µg·L-1).

In order to study the fate of Co in a biological environment, incubation experiments were carried out. Different cobalt concentrations were incubated with two major blood components (HSA, hemoglobin), as well as blood plasma and lysate of a healthy volunteer. Generated Co protein adducts were investigated by means of speciation analysis. The use of HPLC-ICP-MS enabled the separation and detection of different species. A positive correlation of the occurrence of generated species with an increasing concentration of cobalt was observed. The detected species with HSA and the β-subunit of hemoglobin were subsequently further characterized by means of HPLC-ESI-HRMS. The high mass resolution and mass accuracy offered the assignment of specific structures to the found signals. Finally, the developed method was used to analyze the blood sample of an implant patient. In this case, the presence of a cobalt protein complex with HSA could be detected by means of HPLC-ICP-MS via the comparison of retention times with incubation experiments.

Referenzen

Analysis of Volatile and Semi-Volatile Compounds in Ground Black Pepper by Solid Probe-GC/Q-TOF with Novel High Efficiency EI Source

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Keywords: GC, GC/MS, QTOF

Einleitung
Volatile and semivolatile compounds in food continue to be evaluated for health benefits. One of the beneficial classes of compounds is phytosterols, which could reduce cholesterol by blocking its absorption in the small intestine. Terpenes and alkaloids are known for anti-oxidant and anti-inflammatory properties. These compounds have varying chemical compositions and each presents unique challenges in separation, identification and quantitation

Presented here is an exploration in a sample preparation-free analysis of a food matrix, ground Piper Nigrum L (black pepper), by using a solid probe to thermally desorb volatiles onto GC and using a high resolution Q-TOF equipped with a prototype high efficiency electron ionization source that is capable of ionizing analytes with lower energy for enhanced selectivity and identification.

Experimenteller Teil
Two to three flakes of pre-grounded black pepper were transferred to a micro-vial, which was then inserted into the Thermal Separation Probe (TSP). The TSP was placed into the Multi Mode Inlet (MMI), which then was rapidly heated to volatilize all of the compounds. Chromatography was performed on a 7890 GC equipped with a DB-1HT 30m X 0.25mm X 0.10mm capillary column. Helium as carrier gas was set to 5mL/min during the volatilization phase, and then reduced to 1mL/min for the GC separation.

Data were acquired on an Agilent 7200 series GC/Q-TOF equipped with a prototype high efficiency EI source in TOF mode.

Ergebnisse und Diskussion
The prototype high efficiency EI source (HES) can be tuned and operated at different ionization energies to give softer ionization. Increased molecular ion, reduced fragmentation and spectral shift from lower mass fragments to higher mass fragments combined with the power of accurate mass allow for more enhanced selectivity and identification. Figures show spectra, obtained at different ionization energies.

Referenzen

GC-Orbitrap: A new hyphenation for the analysis of complex mixtures

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Keywords: GC-Orbitrap MS, crude oil, Electron Ionization

Einleitung
Crude oil is one of the most complex mixtures in the world and its analysis continues to be a challenging task. Traditional instrumentation usually requires a choice to be made between mass resolving power or a powerful chromatographic separation which ideally enables the distinction of structural isomers which is not possible by mass spectrometry alone. With the recently introduced coupling of a gas chromatographic separation to an ultra-high resolving Orbitrap mass spectrometer both features can now be combined for the analysis of highly complex mixtures, thus enabling a deeper understanding of the samples constituents on a molecular level.

Experimenteller Teil
Light Arabian crude oil was diluted in toluene and separated by gas chromatography on a TRACE 1300 GC Series gas chromatograph (Thermo Fisher, Bremen, Germany) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher, Bremen, Germany). Conditions for the GC separation were as follows: injection: 1 µL split/splitless (60 s), 300 °C; heating: 50 °C (0 min) – 10 °C/min – 300 °C (5 min); carrier flow: constant, 4 ml/min, He; column: 10 m VF5,Varian. Mass spectrometric analysis was performed in full scan mode (m/z 50 – 700) at a resolution of 120 000 (FWHM at m/z 200) using electron ionization (EI) ionization at either 10 eV or 70 eV.

Ergebnisse und Diskussion
The newly developed Orbitrap based GC-MS system provides a high-performance approach for the characterization of hydrocarbons in very complex mixtures such as crude oil. Selection of different ionization energies helps matching low abundant molecular ions to the structurally important fragment signals. The results obtained with hard (70 eV) and soft (10 eV) EI are compared and discussed.

High-energy electron ionization results in significant fragmentation. Although, information about the fragments can be obtained, information about the molecular ions is mostly missing. In addition, chemical identification is problematic due to not only high spectrum complexity but also high background signal.

Low-energy EI can provide information of molecular ions. Under softer conditions mostly hydrocarbons were observed in the present light crude oil. Overall an intensity drop is observed, which can be attributed to lower ionization efficiency. This also explains the low abundance of signals of heteroatom substituted analytes as those are of relatively low overall concentration.

The ability to combine results of different ionization energies provides complementary information on both the molecular ions and fragments which can improve the structural elucidation.

Referenzen

Of Free Ions and Clean Rods – A Study of Contamination in Ion Guides and How to Reduce It

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Keywords: Instrument robustness, contamination reduction, slit Q0 ion guide

Einleitung

Triple quads are the standard in quantitative MS today and robustness is one of their key qualities. The most robust GC/MS designs use Q0 RF-only ion guides as contamination buffers between ion sources and mass analyzers. Patterns observed on ion-guide rods after heavy contamination match the areas where rejected low mass ions hit the rods. This suggests that rejected ions contribute to contamination; either directly by sticking on to the rods reacting with already deposited neutral layers and producing sticky residues.

What if we cut the area of the rods that shows contamination and let the rejected ions out?

Experimenteller Teil

A set of Q0 ion guide rods is modified by cutting slits on the front end of the rods and its performance is investigated through ion simulations and experiments, and compared to a regular ion guide. Experiments are performed on a Bruker EVOQ GCMS triple quad with an EI source. The standard PFTBA calibration compound is used to generate ions used in transmission experiments. Ongoing accelerated contamination experiments are using Methane and Isobutane as CI reagents. Simulations are run using the SIMION 8.1 package (SIS Inc, Ringoes, NJ 08551, www.simion.com) and LUA programming.

Ergebnisse und Diskussion

Experimental transmission curves in terms of ion intensity as a function of RF voltage are compared in for the slit and the regular Q0 ion guides for a range of ion masses in the GCMS instrument. Transmission curves are presented as a function of the RF slope (Voltage/mass), which is proportional to the q-parameter of theoretical stability.

Contamination patterns on ion guide rods match the regions where most rejected ions hit, suggesting that rejected ions contribute to contamination. Slits cut into the entry region of the ion guide rods have negligible effect on ion transmission. The slits allow most rejected ions to escape without hitting the rods, potentially reducing contamination. The slit Q0 ion guide functions similarly to a regular ion guide but benefits on robustness.

Referenzen

Posternummer: 88

On the heating of transfer capillaries of electrospray ion sources

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Keywords: atmospheric interface, transport capillary, instrumentation

Einleitung

In atmospheric interfaces of ambient ionization sources like electrospray ionization (ESI) ions are transferred through a small capillary from ambient pressure to low pressure region. The particle transport is governed by electric forces, diffusion and by the gas flow. The gas flow can be laminar or turbulent, is strongly compressible and intensely heated. Its interplay with the ions determines the behavior of the ion source, in particular efficiency and intensity.

At present, however, the gas flow in this systems in not sufficiently described to fully understand the ion transport. Here, we present a theoretical/numerical and experimental study of the fundamental aspects of ion transport in heated capillaries with high pressure difference.

Experimenteller Teil

Numerical simulations and experimental validations are done side by side.

In a simple experiment the vacuum transfer of RhoB ions from a nano-ESI source is directly measured as absolute ion current. We vary parameters like input current, heating, emitter position and ESI voltage.

The numerical simulation describes ions in a heated gas flow. The gas flow is modeled as a mainly one dimensional flow, obeying the compressible Navier-Stokes equation with heating. The transport is modeled considering space charge, ion mobility, molecular and turbulent diffusion. A simple turbulence criterion is used.

Ergebnisse und Diskussion

Experimentally we find transfer rates near unity of the emitted ions with very high ion currents for a special inlet geometry [1]. Such surprisingly high transmission was later also measured for a different geometry in another group.[2] The characteristic behavior of the source is measured as input-output current relation with a fixed maximum current and a temperature characteristic. Specifically we find a wide range of conditions that provide unity transport strongly dependent on the inlet shape.In order to explain this non trivial behavior, we first investigate the behavior of the gas flow through the capillary only. In particular, the heating of the capillary has a strong important on the gas flow and through this on the motion of the ions, which goes beyond droplet desolvation.In a second step the ion transport is simulated assuming different possible regimes of ion transport, specifically considering laminar-turbulent transitions. We find dramatic differences between these two regimes in terms of ion transmission. Further we illuminate the possibility of the flow behavior being coupled to the capillary heating.A full description of the ion transport includes space charge and diffusion effects. This advanced description is capable of a quantitative prediction of transfer characteristics like in-out current function, maximum absolute current or transmission.Our work sheds light on how and why near perfect transmission is possible. We can therefore provide guidelines for building highly efficient sources, which opens new possibilities for MS applications, which rely on high ion currents or efficient material use, like preparative mass spectrometry [3].

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Posternummer: 89

On-line Biosensor-MS: New Tool for Simultaneous Detection, Structure Determination and Affinity Quantification of Protein-Ligand Interactions

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Keywords: On-line SPR-MS, BIA-MS, Epitope Determination

Einleitung

Analysis of biomolecular interactions using biosensors such as surface plasmon resonance (SPR) or surface acoustic wave have become an established method for detection and quantification of bioaffinity systems. However, the lack of providing structure information of affinity-bound ligands is a major limitation of such analytical procedures. Using an SPR-biosensor for development of an on-line biosensor-MS combination with ESI-MS achieved the simultaneous chemical structure determination and affinity quantification of biopolymer ligands. Key tool of the biosensor-MS combination is an integrated microfluidic interface allowing sample concentration and on-line desalting prior to MS analysis. The broad application areas amenable with the on-line biosensor-MS include affinity-based biomarker identification, identification of protein and peptide epitopes, precise antibody affinity determinations, and direct label-free antigen quantification.

Experimenteller Teil

For validation of the analytical accuracy of the new on-line interface, direct analysis of horse heart myoglobin (HHM) by both mass spectrometry and SPR-biosensor was compared to analysis through the microfluidic interfaceAnti-myoglobin antibody was immobilized on a dextran-SPR chip, as well as on a SepharoseTM micro-column. KD determination between myoglobin and the corresponding anti-myoglobin antibody was performed by investigation of the protein-antibody interaction by direct biosensor study, followed by ESI-MS analysis after sample processing through the interface: Subsequent on-line desalting of analyte prior to MS detection was performed after elution of affinity captured HHM from the integrated affinity column. Comparative data was recorded by straight analysis of the protein in each separate instrument.

Ergebnisse und Diskussion

Following the analysis of HHM and anti-myoglobin antibody separately by both mass spectrometry and SPR biosensor analysis, the protein interaction and molecular identification was carried out by using the microfluidic interface.

Myoglobin is a well characterized protein, with molecular masses of 17558,132 Da or 16941,955 Da for each holo- and apo-protein respectively. Native HHM spectra were recorded under physiological pH in order to compare them to biosensor analysis conditions. Subsequent MS revealed denaturing sample processing conditions: HHM samples were eluted from the affinity column and desalted with 0.1% TFA before ESI-MS analysis. The recorded spectra presented the apo-myoglobin with charge distributions of [M+14H]14+ to [M+24H]24+ showing the denatured protein (without heme-group).

Kinetic studies of the affinity interaction from the biosensor with and without interface coupling were performed. After off-line antibody immobilization, the KD values obtained were in comparable µM ranges both obtained through direct analysis and on-line SPR-MS, proving the validity of the procedure: Formation of myoglobin anti-myoglobin antibody complexes exhibited strong affinities with a KD of 8.99M. The lowest detection limit found during on-line analysis was determined to be in the low micro-molar range.

The new SRP-MS system was able to detect the affinity interaction of the myoglobin ant-myoglobin antibody pair in real time and the recorded spectra showed the apo-myoglobin after interface processing.

The reliability of the interface was established by repeatable and comparable KD determinations and precise mass spectrometric identification of protein. Fast on-line sample processing allows fast throughput of different analytes for biomolecular interaction screenings.

Referenzen

Posternummer: 90

A High Resolution Accurate Mass Approach for the Quantitation of Buprenorphine and Paroxetine in Rat Plasma

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Keywords: quantitation, drug discovery, Orbitrap

Einleitung
As potential leads for successful drug candidates move through the drug discovery process, there is a need for quantitative in vitro and in vivo analysis at each step of the journey. The requirement for quantitative assays that provide sensitivity, ruggedness, and linear response has remained a constant, but as mass spectrometer technology has progressed over time other considerations also play an important role [1]. Ease of use, simplified method development and the availability of troubleshooting tools now represent important factors when choosing the most appropriate technology for a particular assay and the corresponding method development. In this experiment, we examine the quantitative performance of Buprenorphine and Paroxetine in rat plasma utilizing high resolution accurate mass mass spectrometry.

Experimenteller Teil
Protein depleted plasma stock solutions were prepared using an Acetonitrile (ACN) crash at a ratio of 3:1, ACN to plasma. Stock solutions of Buprenorphine and Paroxetine at 1mg/mL were diluted in the crashed plasma stock at concentration ranges from 10pg/mL to 100ng/mL. Chromatographic separation was achieved using a Thermo Scientific™ Vanquish™ UHPLC System. Samples were injected (5uL) onto a 2.1 x 50mm, 1.9um Thermo Scientific™ Hypersil GOLD™ aQ column. Gradient elution was accomplished using water + 0.1% formic acid (FA) (A) and acetonitrile + 0.1% formic acid (FA) (B), with a 6 minute gradient at a flow rate of 500uL/min. Compounds were analyzed on a Thermo Scientific™ Q ExactiveTM Focus MS with heated electrospray ionization.

Ergebnisse und Diskussion
The main goals for any rugged quantitative assay are reaching the lowest possible limit of quantitation, providing a linear response, and maintaining good reproducibility. To evaluate the performance and reproducibility of the LC/MS method, calibration curves were analyzed with replicates of n=6. Linearity and reproducibility were calculated across the working range of the curve. The limit of quantitation (LOQ) was defined as the lowest concentration level that is both within <20% difference of the linear fit and <20% RSD for each group of replicate concentration points. Using the above criteria, the LOQ was determined to be 10pg/mL for both Buprenorphine and Paroxetine. A linear response was observed across four orders of magnitude for Buprenorphine and Paroxetine ranging from 10pg/mL to 100ng/mL. The chromatographic peak width for each analyte ranged between 3.6 and 4.2 seconds at the base, providing 11-15 scans for the target analytes at the LOQ. Selectivity is another criterion for screening or quantitative LC-MS assays [2]. Therefore, the spectrum of each analyte was examined to identify possible interferences. At the resolution setting of 70,000, all interferences present in the sample matrix were adequately resolved from the analyte of interest. These data interrogation options offer valuable method development and troubleshooting tools that provide greater confidence in the analytical method and quantitative results.

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Argentation chromatography coupled with ultrahigh-resolution mass spectrometry for effective separation of heavy crude oil

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Organisation(en): Max-Planck-Institut für Kohlenforschung, Deutschland

Keywords: Heavy crude oil, chromatographic separation, ultrahigh-resolution mass spectrometry

Einleitung

Crude oil is known as one of the most complex mixture and therefore, their analysis even with ultrahigh-resolution MS often requires additional separation steps for simplification. (1) Simplification makes it possible to achieve more detailed information due to the lower amount of different species. Less ion-ion interaction result in higher sensitivity. Argentation chromatography is a good method of choice for separation of heavy crude oils. Here, the separation can be achieved based on the different interaction between the silver ions (Ag+) immobilized on the silica gel surface and the pi-bonds of the different aromatic compounds. The efficiency of the separation coupled with ultrahigh-resolution mass spectrometry was investigated.

Experimenteller Teil

Argentation chromatography was performed on UltiMate 3000 HPLC system. The stationary phase (silver(I)-mercaptopropano silica gel) is not commercially available, therefore it was synthesized in our laboratory using the procedure reported by Nocun et al. (2) Different silica gels were used as starting materials with a particle size of 10 µm (LiChrosorb Si 100 (Merck) and 5 µm (Polygosil 100 (Macherey-Nagel), respectively. The resulting material was packed into a stainless steel column (250 mm x 4.6 mm and 250 mm x 2.1 mm). A mixture of cyclohexane and chloroform was used as mobile phase with stepwise increasing amount of isopropanol to elute the compounds with diverse aromaticity.

MS analysis was performed on a research-type LTQ-Orbitrap Elite MS using APPI ionization technique.

Ergebnisse und Diskussion

In this study argentation chromatographic method was optimized for separation and simplification of heavy crude oil as a complex aromatic mixture. In argentation chromatography the separation takes place according to the different interaction between the aromatic hydrocarbons and/or heterocycles and the silver ion, based on the coordination of the different pi-systems and heteroatoms. The coupling with ultrahigh-resolution MS provides detailed information throughout the whole separation on a molecular level. The efficiency of this separation can be demonstrated by selecting different time ranges of the separation meaning different amount of competitive solvent (isopropanol) and comparing the mass spectra in selected mass windows. The properties of the compounds from the different sections of the separation are discussed in this presentation.

The importance of the characteristic of the stationary phase and the column is noteworthy here. Silver(I) modified silica gel with 5 µm particle size and 250 mm x 2.1 mm column resulted in sharper chromatographic peak shapes in mass spectra and higher sensitivity compared to the one with 10 µm size (packed into 250 mm x 4.6 mm column). Measurement with higher sensitivity makes it possible to detect compounds with high double bond equivalent (DBE), which are not easily ionized and are suppressed otherwise. Specific properties of the compounds at different separation steps will be discussed.

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DNA Oligonucleotide Fragmentation in Collision Induced Dissociation Mass Spectrometry

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Organisation(en): 1: Humboldt-Universität zu Berlin; 2: Bundesanstalt für Materialforschung und -prüfung

Keywords: Collision induced dissociation (CID), Oligonucleotide, Fragmentation

Einleitung
In nucleotides the individual charge state is known to have a great influence to the observed fragment formation. In this comprehensive study relatively small oligonucleotides were used, serving as a test bed for their more complex analogues. We used 15-, 24-, 32- and 40-mers DNA oligonucleotides with a guanine/cytosine content ranging from 0-100 % and different sequences to cover possible sequence and length dependency of the fragmentation characteristics. All oligonucleotides were analyzed by CID in a linear ion trap with helium as collision gas.

Experimenteller Teil
Oligonucleotides in various lengths were used for tandem MS experiments. These were performed in a linear ion trap mass spectrometer LTQ (Thermo Finnigan, San Jose, CA, USA). A concentration of 10 µM solutions of oligonucleotides in water with 30 % methanol and 1% triethylamine was directly infused for MS experiments. The precursor ions were isolated for MS/MS experiments with an isolation width of 2 Dm/z and fragmented with an activation time of 30 ms, while the relative collision energy was varied between 5 and 20 %. Both, the relative collision energy threshold for quantitative fragmentation as well as the corresponding fragmentation pattern were recorded and analyzed.

Ergebnisse und Diskussion
Throughout the study, four different 4-mers, eight 15-mers, five 24-mers, five 32-mers and five 40-mers including different sequences and guanine/cytosine contents ranging from 0 to 100 % were used. All respective oligonucleotides were analyzed systematically in order to identify their individual threshold fragmentation energy in CID experiments. Initially, each monoisotopic signal of the charge series was isolated and then the relative collision energy was varied in 0,1 % steps until fragment ions appeared. The collision energy onset at which fragmentation occurs could be derived at least for eight charge states of the 15-, 24-, 32-, and 40-mers. The results show a strong linear correlation between the obtained threshold collision energy and the respective negative charge density in the dissociating nucleotide. With regards to the different lengths of the oligonucleotides, no significant change in the fragmentation behavior appears to be present. A comprehensive comparison in fact reveals, that the relative fragmentation point is independent to length or sequence.

For all studied nucleotides the collisional threshold energy linearly depends on the charge-to-basepair coulomb density. At intermediate charge densities a minor deviation from linearity in the form of a saddle point can be observed. The existence and position of this saddle point also is independent of the exact sequence and, thus, appears to be the consequence of a general phenomenon. Summing up, the only dependency for oligonucleotide fragmentation appears to be the charge state or the charge per nucleo base ratio, respectively.

Referenzen

High Performance Thin Layer Chromatography Mass Spectrometry using an elution-based TLC-MS interface

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Organisation(en): 1: Merck, Darmstadt, Germany; 2: University of Hamburg, HAMBURG SCHOOL OF FOOD SCIENCE, Hamburg, Germany

Keywords: thin layer chromatography, mass spectrometry, TLC-MS interface, instrumentation, sample preparation

Einleitung

A straightforward way to couple thin layer chromatography (TLC) with mass spectrometry (MS) is the TLC-MS Interface from Camag. It is an elution-based, semi-automatic system to extract zones from the TLC plate and transfer them online into the MS. It is suitable for all thin layer materials and every eluent that can be sprayed in the ion source. The interface can be connected to any kind of LC-coupled mass spectrometer.

Experimenteller Teil

We show how the TLC-MS interface can be used for the development of TLC-MS applications in the areas of food & beverage, pharmaceutical ingredients, cosmetic actives and peptide & protein analysis.

- separation and identification of insulin species

- investigation of UV-filters in suncream

- analysis of steroids

- determination of caffeine in energy drinks

All experiments were performed on newly developed HPTLC plates with a reduced separation layer thickness. After chromatographic separation the analytes were extracted with acetonitrile/water (95:5, v/v) and transferred online into the MS with a flow rate of 0.2 ml/min. The ionization mode was electrospray ionization (ESI) in the positive mode.

Ergebnisse und Diskussion

Thin layer chromatography allows sample preparation and chromatographic separation in one step. This is possible because of the high sample matrix tolerance of TLC. Through the coupling of TLC with mass spectrometry (TLC-MS) substance identification is possible.

It is shown that the use of thinner TLC plates leads to improved detection limits, increased sensitivity and improved S/N ratios. The sample matrix is clearly separated from the target analytes. This leads to clean mass spectra due to a very low level of ion suppression.

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[3] Morlock, G.E., Background mass signals in TLC/HPTLC-ESI-MS and practical advices for use of the TLC-MS interface, J. Liq. Chromatogr. Rel. Techn., 37, 2892-2914, 2014

IMPROVED DETERMINATION OF ALLERGENIC FRAGRANCES IN DETERGENTS AND PERSONAL CARE PRODUCTS IN MULTIPLE REACTION MONITORING GC-MS

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Organisation(en): Bruker Daltonik GmbH, Deutschland

Keywords: allergens, detection, fragrance, GC, Triple Quad

Einleitung
Sensitization, intolerance and (pseudo)allergenic reactions represent contrary answers to perfume and fragrances.

Experimenteller Teil
According to EU Directive 76/768/EC a number of allergens should be labeled if they exceed a certain concentration. The concerning allergens are mentioned in Annex III ofRegulation (EC) No 1223/2009. Regarding the detergents directive (EC) no.648/2004 allergens should be labelled as stated in the cosmetic regulation from0.01%(w/w).

Ergebnisse und Diskussion
In this study suitable transition have been studied to identify >20 compounds moreaccurate by multiple reaction monitoring.

Referenzen

Posternummer: 95

Mass spectrometry of peptides using a combination of thin-layer chromatography and desorption/ionization induced by neutral SO2 clusters

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Organisation(en): 1: Justus-Liebig-Universität Gießen, Deutschland; 2: Bruker Daltonik GmbH, Bremen, Deutschland

Keywords: Matrix-free soft desorption/ionization, thin-layer chromatography, peptides, cluster-induced desorption

Einleitung

Thin-layer chromatography (TLC) is a common method to separate complex mixtures and can be performed with a simple setup. For identification of the separated components thin-layer chromatography can be combined with different mass spectrometry techniques [1]. Desorption/ionization induced by neutral SO2 clusters (DINeC) is an extremely soft and matrix free ionization method for mass spectrometry [2,3]. In this work, the compatibility of TLC and DINeC was investigated using mixtures of different oligopeptides. Such test samples were successfully separated by TLC and analysed by means of DINeC-MS without any further sample treatment.

Experimenteller Teil

Solved Oligopeptides were mixed and drop cast on a thin-layer chromatography plate which is composed of an aluminum plate and a silica gel layer as stationary phase. After drying, TLC was performed with a mixture of water, 2-butanol, ammonia and pyridine as mobile phase. After separation by means of TLC, the plate was attached to the sample holder of the DINeC apparatus. The holder was moved stepwise through the beam of neutral SO2 clusters (mean cluster size 103 to 104 molecules, seeded in He, beam diameter approx. 5 mm). The analytes were desorbed and ionized by the impacting clusters [2,3]. Mass analysis of the desorbed ions was performed in a Bruker Amazon ion trap mass spectrometer.

Ergebnisse und Diskussion

First experiments showed that angiotensin II, bradykinin, vancomycin, and bombesin molecules can be efficiently desorbed and ionized from the thin-layer chromatography plates by means of DINeC. The [M+H]+ signal of the respective substance is clearly detected. Stepwise scan of the thin-layer chromatography plate allows to measure mass spectra in dependence on the position. Extracting the signal height of the [M+H]+ peak for every substance in the mixture, a TLC chromatogram of the single components as identified by DINeC-MS is obtained. The separated analytes reveal different intensities while the amount of sample molecules is identical. Nevertheless, it is possible to identify close bands due to the shape of the intensity profile. This demonstrates that thin-layer chromatography and DINeC can be successfully combined. The cluster beam is small enough to provide a satisfying spatial resolution and the amount of molecules which are desorbed and ionized out of the porous TLC plate is sufficient. As no further sample treatment such as extracting the analytes for ESI-MS or application of a matrix for MALDI is necessary, the analysis of oligopeptides with these two techniques requires very little effort in sample preparation. The application to vancomycin further demonstrates the soft nature of DINeC also in combination with thin-layer chromatography.

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Posternummer: 96

Multivariate Datenanalyse in der Massenspektrometrie: Fallbeispiele zum Einfluss der Datenvorbehandlung

Autoren: Heller, Danica (1,2); Albert, Anastasia (3); Ebert, Franziska (4); Leffers, Larissa (4); Schwerdtle, Tanja (4); Hagenhoff, Birgit (1); Engelhard, Carsten (2)

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Keywords: Multivariate Datenanalyse, Massenspektrometrie, Datenvorbehandlung

Einleitung
Multivariate Datenanalysen (MVA) haben für Auswertung massenspektrometrischer Daten eine immer größere Verbreitung gefunden. Da die Aussagefähigkeit der Ergebnisse von MVA-Anwendungen von der Datenvorbehandlung abhängt, kommt diesem Schritt eine große Bedeutung zu.1,2 Häufig werden zunächst Standardmethoden zur Vorbehandlung eingesetzt. In dieser Arbeit wird anhand von drei verschiedenen Beispielen demonstriert, dass eine an die Problemstellung angepasste Datenvorbehandlung jedoch zu deutlich besseren Ergebnissen im Hinblick auf die analytische Ausgangsfrage führt. Als massenspektrometrische Techniken werden die Flugzeit-Sekundärionen-Massenspektrometrie- (ToF-SIMS) und die Kaltplasma-Massenspektrometrie (LTP-MS) eingesetzt.

Experimenteller Teil
Für die Untersuchungen wurden drei unterschiedliche Probensysteme verwendet. Das erste Probensystem besteht aus Anoden formierter Li-Ionen-Batterieproben (LiBs), denen verschiedene Additive (Ethylensulfit, Fluoroethylencarbonat, Vinylencarbonat) zugefügt wurden, um das Alterungsverhalten der LiBs zu beeinflussen.3 Das zweite Probensystem enthält entsprechende Anodenproben in verschiedenen Alterungszuständen (kalendarisch gealtert, formiert, gealtert). Von allen Proben wurden jeweils 5 ToF-SIMS Oberflächenspektren aufgenommen.
Für die LTP-MS Untersuchungen wurden Proben aus verschiedenen menschliche Krebszelllinien (Lungenepithelzellen, Leberkarzinomzellen, Urothelialtumorzellen) präpariert: Die Zellkulturen wurden in entsprechenden Nährmedien angelegt und unter Standardbedingungen kultiviert. Für die Validierung wurden zusätzlich Proben aus reinem Nährmedium angelegt. Von den Zellen und Nährmedien wurden jeweils 5 LTP-MS Spektren aufgenommen.
Die Spektren der drei Probensysteme wurden nach unterschiedlicher Datenvorbehandlung mit Hilfe der Hauptkomponentenanalyse (PCA) analysiert.

Ergebnisse und Diskussion
Im ersten Probensystem (LiBs) wurden verschiedene Zentrierungen und Normierungen verglichen, um die Methode zu finden, die die Einflüsse der zugesetzten Additive auf die Oberfläche der Batterieanoden bestmöglich beschreibt. Es wird gezeigt, dass eine an der Fragestellung ausgerichtete Zentrierung gegenüber anderen Standardzentrierungen, wie der Mittelwertzentrierung, überlegen ist.

Im zweiten Probensystem (gealterte LiBs) wird mit der gleichen Zentrierung wie im obigen Beispiel zwar die größte Varianz im Datensatz (d.h. Unterschiede zwischen den verschiedenen Alterungsstufen) von der PCA aufgeschlüsselt, der Einfluss der Additive wird allerdings besser mit Hilfe einer nochmals angepassten Zentrierung ermittelt.

In dem weiteren Beispiel der LTP-MS Daten von Krebszellen wird zunächst die häufig genutzte Autoskalierung zur Datenvorbehandlung verwendet. Es wird gezeigt, dass diese Skalierungsmethode in diesem Fall ungeeignet ist. Eine passendere Skalierung ermöglicht eine eindeutige Trennung der verschiedenen Zelllinien von ihrem Nährmedium sowie die Unterscheidung einzelner Zelllinien. Eine Erklärung dafür liefert die Analyse von verschiedenen Ansätzen der gleichen Zelllinie. Diese zeigt, dass nach Autoskalierung das Rauschen signifikant zur MVA-Modellbildung beigetragen hat.

Insgesamt wird gezeigt, dass die Ergebnisse und die Qualität von MVA-Ergebnissen im Bereich der Massenspektrometrie stark von der Datenvorbehandlung abhängig sind. Um die Vorzüge der MVA (Vereinfachung sehr komplexer Datensätze, Minimierung der benötigten Vorinformation über das Probensystem) auszunutzen, muss für jedes System die passende Datenvorbehandlung gefunden werden. Es wird deutlich, dass eine problemorientierte Datenvorbehandlung, die anhand des Probensystems problemorientiert validiert wird, den standardmäßig eingesetzten Datenvorbehandlungen überlegen ist.

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Posternummer: 97

Rapid Autoxidation of Squalene Aerosol Particles probed by Aerosol Mass Spectrometry

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Organisation(en): Chemical Sciences Division, Lawrence Berkeley National Lab, Berkeley, USA

Keywords: aerosol mass spectrometry, atmospheric chemistry, organic aerosol, oxidation, ion mobility

Einleitung

As the most abundant unsaturated constituent of skin lipids, squalene plays an important role for indoor air quality, but also serves as a proxy for unsaturated hydrocarbons relevant in combustion and atmospheric chemistry. Once in contact with air, photochemical transformation readily occurs, e.g. via reaction with O3 or OH, leading to fragmentation, isomerization and/or functionalization. Hence, chemically changed species are formed which have been shown to constitute a health hazard in numerous cases.[1] While ozonolysis of squalene has been studied intensively, little is known about kinetics, reaction pathways and product formation of OH uptake, in particular at environmental conditions.

Experimenteller Teil

In order to create such conditions, polydispers liquid squalene particles are introduced into a continuous-flow stirred-tank reactor. Here, the steady state can be hold for an arbitrary duration, allowing for low concentration measurements up to several hours. In combination with a VUV photoionization aerosol mass spectrometer this set up allows monitoring the kinetics of an OH-initiated reaction and to determine the chemical composition of an aerosol in the course of the reaction.[2] In order to get a deeper insight into the nature of the products, the reaction has also been probed using a Q Exactive Orbitrap Mass Spectrometer (Thermo Scientifc) with different atmospheric pressure ionization sources (ESI, DART), and in combination with an ion mobility drift cell (HPIMS, Excellims).

Ergebnisse und Diskussion

The results show that the effective uptake coefficient increases as a function of decreasing OH concentration ([OH]). While at high [OH] (2·108 molec./cm3) chain termination reactions involving OH radicals result in an effective uptake coefficient of 1.9, particle-phase secondary chain chemistry prevails at environmental [OH] (~105 molec./cm3) thus increasing the effective uptake to over 100. The reaction appears to form highly oxidized multifunctional reaction products with O/C ratios greater than 0.6. Preliminary analysis based on the identification of masses with different types of mass spectrometry and comparison to a stochastic kinetics model suggest that these products are multifunctional hydroxyperoxyalkyl radicals, formed via inter- or intramolecular H-abstraction, which further react to stable hydroxyperoxides (QOOH). The effects of QOOH on indoor air quality and skin are yet unknown, however the structurally similar squalene peroxide (formed outside via UVA/UVB and photoxidizer) has been shown to cause inflammatory skin diseases such as skin cancer and skin ageing.[3] In the atmosphere QOOH may play a signficant role in promoting the formation of new particles, which furthermore can act as cloud condensation nuclei.[4]

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Identifizierung unbekannter Substanzen in SIMS-Spektren - Multivariate Datenanalyse zur Beschreibung wenig charakterisierter Datensätze

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Organisation(en): 1: Tascon GmbH, Deutschland; 2: Universität Siegen

Keywords: Multivariate Datenanalyse, Flugzeit- Sekundärionen-Massenspektrometrie, Oberflächenanalyse

Einleitung
Mit Hilfe der Flugzeit-Sekundärionen-Massenspektrometrie (ToF-SIMS) ist es möglich, die organische und anorganische Zusammensetzung von Oberflächen simultan zu analysieren. Dies geschieht mit hoher Lateral- und Tiefenauflösung, so dass detaillierte Sekundärionenbilder, Tiefenprofile oder 3D-Datensätze aufgenommen werden können. Die ToF-SIMS ist besonders für die Analyse von Probensystemen mit unbekannter Zusammensetzung geeignet. Die erhaltenen Spektren sind jedoch sehr komplex. Die Auswahl relevanter Peaks und die Identifikation der chemischen Zusammensetzung ist für diese Probensysteme sehr zeitaufwendig. In dieser Studie wird die Peakidentifizierung durch die Anwendung Multivariater Datenanalyse (MVA) vereinfacht. Zusätzlich ermöglicht die MVA die Analyse der Probenzusammensetzung in mehreren Dimensionen (Oberfläche und Tiefeninformation).

Experimenteller Teil
Das Probensystem besteht aus Li-Ionen-Batterieanoden (LiBs), denen verschiedene filmbildende Additive (Ethylensulfit, Fluoroethylencarbonat, Vinylencarbonat) zugesetzt wurden, um die Alterungseffekte bei der Zyklisierung (Belastung durch Lade- /Entladezyklen) zu beeinflussen. Mittels Tof-SIMS (ToF.SIMS5, IONTOF, Münster, Deutschland) wurden jeweils fünf Oberflächenspektren sowie zwei Tiefenprofile von einer Anode nach Zyklisierung in einer Testzelle aufgenommen. Die Auswertung der Spektren erfolgte mit Hilfe der Hauptkomponentenanalyse (PCA), für die Tiefenprofile wurde die Multivariate Curve Resolution (MCR) verwendet. Anschließend wurde eine weitere PCA auf die MCR Ergebnisse angewendet.*Ergebnisse und Diskussion*
Diese Studie zeigt am Beispiel von Batterieanoden einen Ansatz für ein generelles Vorgehen zur Analyse von Probensystemen mit unbekannter Zusammensetzung. Im ersten Schritt wurden die Oberflächenspektren mit Hilfe der PCA klassifiziert. Dabei kann eine Trennung der verschiedenen Additivproben beobachtet werden. Durch eine an die ToF-SIMS angepasste Ausführung gelingt die Selektion relevanter Signale sowie die chemische Identifikation von ca. 76 % der Signale mit zuvor unbekannter Zusammensetzung. So konnten u.a. Zersetzungsprodukte des Elektrolyten erfolgreich identifiziert werden. In den Tiefenprofilen wurden zusätzliche Signale beobachtet, welche mit Hilfe der PCA-Ergebnisse leichter identifiziert werden konnten. Mit Hilfe der MCR Analyse war es möglich, den Schichtaufbau der Anodenproben mit Deckschicht (Elektrolytreste, Verunreinigungen), Solid-Electrolyte-Interface Schicht (SEI) sowie Elektrodenmaterial zu charakterisieren. Eine Abhängigkeit der SEI-Schichtdicke von den eingesetzten Additiven zeigte sich nicht. Ein Einfluss eines Additives auf die Schichtzusammensetzung konnte nur für die Probe mit dem Additiv Ethylensulfit nachgewiesen werden (Sulfitnachweis in der SEI-Schicht). Für alle anderen Proben wurde eine vergleichbare Zusammensetzung der einzelnen Schichten gefunden.

Diese Arbeit liefert einen Ansatz für automatisierte MVA: Die manuelle Peakauswahl entfällt und eine Vorinformation über das Probensystem wird entbehrlich. Um eine vollständig automatisierte Datenauswertung zu gewährleisten, müssen in Zukunft allerdings allgemein gültige Richtlinien für eine passende Datenvorbehandlung (Skalierung, Zentrierung, Normierung) entwickelt werden.

Referenzen

Posternummer: 99

De novo synthesis of glycosaminoglycans during tenogenesis studied by matrix-assisted laser desorption/ionization mass spectrometry

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Keywords: MALDI TOF MS, Glycosminoglycans (GAGs), 13C labeling

Einleitung

Tendon injuries are the most occurring musculoskeletal disorders in vertebrates. The tendon healing capacity in vertebrates is limited. Lately successful healing could be observed by the medical treatment with autologous mesenchymal stem cells (MSC).

To improve tendon therapies and avoiding at least the extension of animal experiments, tendon equivalents get generated ex vivo using a bioreactor.

The mechanisms of tenogenesis are not well understood, therefore the investigation of the components of de novo generated tendon, mostly collagen and glycosaminoglycans (GAGs), is crucial. Here we show that MALDI TOF MS is a certain technique to monitor GAG synthesis in bioengineered tendon.

Experimenteller Teil

Mesenchymal stromal cells were isolated from equine adipose tissue. Cells were detached, washed and cultivated in DMEM (without glucose) plus 13C labeled glucose for different time points after a “5-day-preliminary-cultivation”.

After the cultivation, cells were resuspended in 90 µL H2O and 0.2 units of chondroitinase ABC per vial. The enzymatic digestion was performed overnight (12h) at 37°C.

All mass spectra were acquired on a Bruker Autoflex mass spectrometer equipped with a 377 nm pulsed nitrogen laser. The measurements were performed in the reflector mode, 9-AA (10 mg/mL in 2-propanol/acetonitril, 60/40, v/v) was used as matrix for negative ion detection.

The internal standard, analyte and matrix were mixed 1/3/2, v/v/v prior to the application. All spectra were evaluated using FlexAnalysis (3.0).

Ergebnisse und Diskussion

The incorporation of 13C isotopes during the biosynthesis of GAGs leads to higher molecular masses. In this way the monitoring of de novo generated GAGs is possible. There is a correlation between time and the incorporation of 13C isotopes. A major enhancement occurs between one and two weeks of cultivation. It can be shown that MSC use intact glucose for GAG synthesis.

The signals at m/z 464 and 470 indicate the inclusion of one uronic acid residue, more precisely both sugar rings (completely 13C labeled glycopyranose units).

The inclusion of the 13C labeled N-acetyl group is indicated by the signal at m/z 472. It means in effect, the unsaturated disaccharide does not contain 12C atoms. Furthermore, peak assignments were checked by post source decay (PSD) MS.

Moreover, it is clear, that partial 13C labeling of the glycopyranose units takes place, which is shown due to the presence of smaller m/z values. These results suggest that GAG syntheses can be smoothly studied by MALDI TOF MS. By comparison with the signal intensities of the non-sulphated disaccharide or native unsaturated disaccharide (C0S, m/z 378; CS, m/z 458) quantitative statements are possible. Structural assignments from single MSexperiments should be regarded with caution. Since both glycopyranose units contain 6 carbon atoms, the localization of 13C atoms is not possible using only MS. The generation of specific fragment ions enabled an exact characterization of the labeled structure. Nevertheless, a tandem mass spectrometer is not absolutely essential – PSD mass spectra are sufficient.

Briefly, the 13C incorporation provides a solid method for monitoring GAG synthesis from cultivated MSC. The 13C assembly from glucose into other compounds of tendon matrix, e.g. collagen, could not be observed. This strongly suggests that 13C labeled glucose is solely integrated into GAGs.

Referenzen

Differentiation of chondroitin sulfate isomers by ion mobility spectrometry-mass spectrometry and nuclear magnetic resonance spectroscopic diffusion measurements

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Keywords: Chondroitin Sulfate, Disaccharide, Ion Mobility Spectrometry-Mass Spectrometry, NMR, Apparent Self-Diffusion Coefficient

Einleitung

Glycosaminoglycans (GAG) such as chondroitin sulfate (CS) are important constituents of the extracellular matrix and play an important role in all vertebrates [1].

Typically MS/MS (subsequent to enzymatic digestion) is utilized to differentiate the naturally occurring isomers (4- and 6-sulfate) of CS. Qualitative and semi quantitative evaluation of the MS/MS results requires standards to compensate the different ionization potentials of the isomers and to determine the most relevant fragments [2].

We were looking for alternative methods which enable the differentiation of isomers without the need of using MS/MS - particularly regarding the future analysis of artificially sulfated GAG-derivatives. For this purpose, isomeric CS disaccharides obtained by chondroitinase ABC digestion served as the compounds of interest.

Experimenteller Teil

Two commercially available CS disaccharide standards and one sample obtained by digestion of the CS polysaccharide with chondroitinase ABC were investigated via ion mobility mass spectrometry (IMS) and nuclear magnetic resonance (NMR) spectroscopy.

Mass spectra were acquired on a TOFWERK IMS-TOF which has been essentially described in [3]. Analytes were ionized by ESI. Nitrogen was used as the drift gas at atmospheric pressure and a temperature of 30°C.

The proton NMR spectra were recorded at 25°C using an inverse 5 mm probe on a Bruker 600 MHz NMR-Spectrometer. Diffusion measurements were performed at 37°. All samples were investigated as about 5 mg/ml solutions in D2O.

Ergebnisse und Diskussion

The negative (deprotonated form m/z 458) and positive (triply sodiated disaccharide m/z 526) ion mobility mass spectra of the digested CS show two signals which are even baseline-separated in the case of the positive ion mode. The IMS-MS spectra of CS standards verify that the first peak, characterized by a shorter drift time, represents the 4-sulfate and the second peak the 6-sulfate.

The differences in the drift time of the two isomers can only originate from the different spatial extensions of the related ions. In detail, the sulfate residue at the C-6 position is more space-demanding in comparison to the same modification at the C-4 position, leading to different collision cross sections.

These findings could be confirmed by 1H pulsed field gradient NMR spectroscopy [4]. The logarithmic plot of I/I0 in dependence on the applied field gradient strengths shows different slopes for the CS isomers. The signal intensity of the resonance assigned to the C4S isomer drops faster, corresponding to a lower apparent self-diffusion coefficient than the C6S isomer.

These data are in excellent agreement with the IMS-MS results. In a nutshell, the CS isomer which is sulfated at the C4-position is “faster” than the C6S-form in both environments, in the gas phase as well as in solution, caused by the conformational differences between the two isomeric forms.

The presence of positive counter ions on the negatively charged sulfate group in positive ion mode increased the difference in the collision cross section, and thus, the drift time, allowing baseline separation of the two signals.

All in all, the differentiation of the two isomers is possible by IMS-MS without the need to perform MS/MS experiments. Additional measurements showed that not only the isomers differ in their drift time but also the location of the counter ions (e.g. Na+) can be estimated.

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Identification of Isomeric Carbohydrates by Collision Cross Section Fingerprinting

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Keywords: Ion mobility-mass spectrometry, carbohydrates, collision cross section

Einleitung

Carbohydrates are of great importance for a variety of biological functions such as cell-cell-recognition, immune response or protein folding. However, compared to proteins or DNA, oligosaccharides are not linearly assembled, but frequently contain branched structures with a complex regio- and stereochemistry. This often results in the presence of isomers which pose a large challenge for carbohydrate analysis, because they can often not be differentiated using mass spectrometry (MS).

Experimenteller Teil

A promising approach to overcome the above-mentioned limitation is to implement an additional gas-phase separation step using ion mobility-mass spectrometry (IM-MS). Here, ions travel through a gas-filled cell aided by a weak electric field and are separated according to their mass, charge, and shape. While the drift time of an ion depends on the underlying instrument conditions, it can be converted into a collision cross section (CCS), which is a molecular property that can be universally compared and calculated theoretically. In addition, IM-MS has the advantages that the analysis time is very short, the sample consumption low, and it can be coupled to HPLC.

Ergebnisse und Diskussion

In a previous study we were able to demonstrate, that carbohydrate linkage- and stereoisomers, which are difficult to distinguish using established techniques can be separated and unambiguously identified on basis of their CCS.[1] More importantly, however, these experiments also showed that larger carbohydrates as well as glycopeptides can be identified using gas-phase fragmentation and subsequent IM-MS analysis. This indicates that IM-MS is an exceptionally effective tool for the structural analysis of oligosaccharides and has the potential to become a standard technique for their sequencing.

Here, we demonstrate that IM-MS can be used to unambiguously identify oligosaccharides when CCSs of precursor and fragment ions are monitored simultaneous. To do so, we systematically investigated common glycan motifs such as Lewis and blood group epitopes and recorded their fragmentation spectra. Our results show that the resulting multidimensional datasets enable structural identifications on basis of combined m/z and CCS fingerprints. The implementation of these data into the open access database GlycoMob will help to vastly improve the analysis and assignment of unknown carbohydrates in the future.[2]

Referenzen

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Lewis-Y Antigens found Dominantly on N-Glycans of Salivary Prolactin-Inducible Protein

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Organisation(en): Universität Hamburg

Keywords: fucosylation; glycoprotein; Lewis antigens; prolactin-inducible protein; saliva

Einleitung

Prolactin-inducible protein (PIP) is a 13.5 kDa single-chain protein showing one N-glycosylation-site. PIP is secreted from exocrine glands and thus found e.g. in saliva, seminal fluid and during pathogenic states of mammary glands. Many important biological functions of PIP have been identified, such as the inhibition of bacterial growth and the involvement in tumor progression. Here, we show the analysis of the N-glycosylation of PIP from different sources by HR-LC-MS(/MS) and NMR. We found a unique N-glycosylation in healthy individuals from seminal fluid and saliva. PIP carries only highly fucosylated N-linked glycans as Lewisy structures. In many organs Lewisy is up-regulated in tumor genesis. These results demonstrate for the first time unambiguously N-type Ley antigens on a specific glycoprotein.

Experimenteller Teil

Whole saliva was collected from healthy individuals and centrifuged. The supernatant was separated by size exclusion chromatography and/or anion exchange chromatography. Intact PIP was analyzed by HR-ESI-q/TOF in positive-ion mode. For removal od sialic acids, lyophilized PIP was redissolved in sodium acetate buffer (pH 5) and incubated with neuraminidase (from Clostridium perfringens, C. welchii). PNGase F was used to release N-glycans, which were purified from peptides by solid phase extraction. Free N-glycans were separated on a PGC (porous graphitized column) column. 5% of the flow was directly injected into the MS and 95% were collected in deep well plates for NMR analyses. 1H- and TOCSY-NMR experiments were carried out for unambiguous confirmation of Lewis-Y structures.

Ergebnisse und Diskussion

We show an unique structural motif of highly fucosylated N-glycans of PIP. PIP was isolated from human saliva and analyzed by HR-LC-MS(/MS) and NMR. In the MS spectra of the intact protein we identified bi-, tri- and tetra-antennary N-glycan structures. All structures carry extremely high amounts of fucosyl (Fuc) residues. For example, the highest intensity in the mass spectra of the intact PIP is representing a triantennary structure with seven fucosyl residues. Similar patterns are found for the biantennary and the tetraantennary structures with the biantennae showing two to five Fuc residues and the tetraantennae two to nine Fuc residues. This represents a before unseen pattern of N-glycosylation from an individual glycoprotein. A similar pattern was also found on PIP from seminal fluid. Two Fuc residues on one GlcNAc-Gal antennae leave two options for the antigenic nature of the terminal tetrasaccharides: Lewis-b and/or Lewis-y. To assign these terminal fucosylated tetrasaccharides, the linkage configuration as well as the attachment points of the fucosyl residues has to be elucidated. Therefore, the N-glycans were cleaved from salivary PIP with PNGase F and separated on a PGC column to perform NMR analyses. To elucidate the fucosylated antigenic structures 1H-NMR and TOCSY spectra were acquired. Signals characteristic for Ley were identified that are in excellent agreement with NMR data of synthetic Ley structures and therefore confirm the unusual presence of Ley epitopes on N-glycans. Ley structures are rarely found on normal cells but are highly expressed in many tumor tissues. However, it is found on seminal and salivary PIP of healthy individuals. This occurrence of Ley antigens on N-glycans needs more research to understand its involvement in tumor progression.

Referenzen

Probing lectin carbohydrate-binding specificity by combining thin-layer chromatography, antibody-overlay detection and desorption electrospray ionisation Fourier transform-ion cyclotron resonance mass spectrometry

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Keywords: Lectins, DESI, FT-ICR, gangliosides, sialic acid

Einleitung

Lectins acting as carbohydrate recognition determinants in diverse biological processes are widely used in a broad range of applications including the detection, isolation and structural studies of glycoconjugates due to their specific sugar-binding properties. In the present study the carbohydrate-binding specificity of a lectin previously isolated from the seeds of Trichosanthes dioica (TDSL) [1] was probed comprehensively. To this end, we made use of a combination of immunodetection of glycosphingolipids (GSLs) [2] acting as high affinity ligands of TDSL and their subsequent accurate and precise analysis by application of desorption electrospray ionisation Fourier transform-ion cyclotron resonance mass spectrometry (DESI FT-ICR MS).

Experimenteller Teil

Binding specificity of TDSL towards GSLs was investigated by thin-layer chromatography (TLC) overlay assays with GSLs from different sources employing an antibody directed against xylose present in pauci-mannose type N-glycans found in TDSL [3]. Subsequently, incubation with an alkaline phosphatase-labelled secondary antibody and staining with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) gave rise to bright blue bands.

For DESI FT-ICR MS measurements, the plate was placed onto a movable sample stage and analytes were desorbed and ionised directly from the plate by use of methanol as a spray solvent. Mass analysis was performed with a Bruker Apex II FT-ICR mass spectrometer equipped with a 7 T magnet.

Ergebnisse und Diskussion

Previous studies suggested a preferred binding of TDSL towards galactose and/or N-acetylgalactosamine [1]. Here, we show that careful analysis of the carbohydrate-binding specificity of TDSL exhibits strong binding to gangliosides comprising α2-6-linked sialic acid as well as weaker interaction with the Galβ1-4GlcNAc motif present in neutral GSLs. Notably, gangliosides harbouring terminally α2-3-linked sialic acid were not recognised by TDSL.

Immunostained bands were analysed by DESI FT-ICR MS and lectin-binding GSLs could be desorbed and ionised directly from the TLC plate without interferences from the lectin, antibodies or the staining reagent. Albeit gangliosides harbour per definitionem acidic sialic acid monosaccharide building blocks ionisation of the analytes in negative ion mode was hampered. However, positive ion mode spectra showed the formation of stable, doubly sodiated molecules which allowed for unambiguous identification of high and low affinity ligands of TDSL. These results indicate that TDSL has to be regarded as a lectin with preferential binding specificity towards oligosaccharides containing terminally α2-6-linked sialic acid and minor affinity towards terminal Galβ1-4GlcNAc moieties. The present study demonstrates the potential of the hyphenation of planar chromatographic separation and formation of gaseous ions directly from the surface of the TLC plate by DESI for the analysis of immunostained GSLs serving as lectin ligands. Knowledge of carbohydrate-binding specificities of lectins is a prerequisite for their use in biomedical applications e.g. as probes for mammalian ganglioside receptors of influenza A viruses.

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Posternummer: 104

Analytical Implications for Conducting Precision Drift Tube Ion Mobility Measurements in Helium, Nitrogen, and Carbon Dioxide

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Organisation(en): Agilent, Deutschland

Keywords: Ionenmobilität

Einleitung

This Poster describes recent improvements in the gas delivery and monitoring system of a commercial drift tube ion mobility-mass spectrometer (Ion Mobility TOF, Q-TOF 6560A)

Experimenteller Teil

Gas specific dependencies on the ion mobility resolving power and other practical considerations for conducting ion mobility experiments in alternative gases are discussed

Ergebnisse und Diskussion

The instrument is capable of operation at the same pressure, temperature and similar fields for all three drift gases investigated, which faciliitatesdirect comparisons between experimental results.

Carbon dioxide and nitrogen access the highest resolving powers. Resolving power was found to depend on the ions reducer mobility, which enabled theoretical mapping of the instrument resolving power.

Higher resolving power does correlate to better separations as observed from measurements obtained on a mixture of three isomeric carbohydrates, but the differences in resolution are subtle.

Helium based CCS measurements are found to correlate better to current theoretical methods

Referenzen

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Comparison of CCS(N2) measurements obtained from two different T-wave IMS systems with direct measurements using a drift tube IMS

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Keywords: CCS, T-Wave, IMS

Einleitung

With recent developments in IMS instrumentation, interest has increased in the determination of collisional cross-sections (CCS) of various classes of compounds. These CCS measurements can be used to augment screening of complex samples both by reducing interfering effects of matrix ions and as an additional identification criterion. Additionally they assist structural confirmation of isoforms / isomers. The widely used T-Wave IMS system relies on calibration to provide CCS values as there is no direct analytical solution for the complex motion of ions through the device. The efficacy of T-wave IMS calibration is investigated by comparison of CCS values obtained from a linear-field drift tube IMS, theoretical calculation and available literature values.

Experimenteller Teil

CCS data were acquired in nitrogen drift gas (CCS(N2)) on two geometrically different calibrated T-wave IMS systems. These data were compared to CCS(N2) values measured on a Synapt G2-Si modified with a linear-field drift tube in place of the standard T-wave IMS device. The CCS(N2) values of selected analytes were also compared to theoretical CCS(N2) values obtained using a nitrogen optimised version of MOBCAL and literature values. Numerous classes of compounds were analysed across all three platforms, delivered via direct infusion and UPLC using electrospray ionisation. The species used for mobility calibration of the T-wave devices was poly-DL-Alanine, introduced by infusion.

Ergebnisse und Diskussion

Over 200 CCS(N2) measurements have been made across various classes of compound (drug like small molecules, sugars, polymers, tetra-alkyl ammonium salts, phosphazines and peptides). Initial data were acquired on the modified Synapt G2-Si and then used as a benchmark for comparison with literature values. Subsequently data were acquired from the two different geometry calibrated T-wave IMS systems and theoretical cross sections were calculated for comparison. Initial results indicate good consistency between CCS(N2) values obtained from the linear-field drift tube and the two calibrated T-wave IMS systems, with RMS deviation of 1.2% for a Synapt G2-Si and 1.3% for a prototype T-wave IMS system . Similarly CCS(N2) values between the two different geometry T-wave IMS systems were good with a RMS deviation of 1.0%. Close agreement of CCS(N2) values were also observed between the prototype T-wave IMS system and CCS(N2) values cited in the literature, with RMS deviation of 1.9%. Comparative accuracy of calibrated T-wave IMS CCS(N2) values to those determined directly on a linear drift tube IMS of species of charge state 2 or higher are improved when a multiply charged calibration is used and this will be discussed further. Results will show long term CCS(N2) reproducibility on the prototype T-wave IMS system along with discussion on methods for improving long term stability. In addition, the comparison of CCS(N2) data with theoretically derived CCS(N2) values will be presented.

Referenzen

Posternummer: 106

Evaluation of the Utility of Alternative Drift Gases in a Low Pressure Conventional Drift Tube Ion Mobility MS

Autoren: Falter, Ralf; Kurulugama, Ruwan; Stafford, Georg; Mordehai, Alex

Organisation(en): Agilent, Deutschland

Keywords: Ion mobility

Einleitung

Helium was used as preferred drift gas in low pressure drift tube ion mobility instruments.

Evaluation of the Utility of Alternative Drift Gases in a Low Pressure Conventional Drift Tube Ion Mobility MS experimentalinly due to the fact that theoretical collision cross section CCS calculations using helium drift gas is relatively well developped and such measurements can be compared with the experimentally obtained CCS values to obtain structural information. In most cases nitrogene is used as a drift gas. Different drift gases are used in this study to obtain high ion mobility resolution.

Experimenteller Teil

A commercial available drift tube ion mobility mass spectrometer 6560 QTOF was used to carry out the experiments. Isobaricc compounds like leucin and isoleucine, raffinose and melezitose, different hexose mono phosphates were tried to spearate by ion mobility. Helium, Nitrogen, N2O, SF6,CHF3 were used as drift gas.

Ergebnisse und Diskussion

The power of Ion mobility resultiom for the isobaric compound was optmized and determined.

The best separation of rafinose and melezitose was obtained by helium dan nitrogen.

The best separation of leucin and isoleucine was obtained by nitrogen.

The best separation for the hexose monot phospates was obtained by nitrogen

Referenzen

[1] J.C.May et al. Anal. Chem., 2014, 86, 2107-2116

High resolution ion mobility-mass spectrometry for the analysis of natural products and complex mixtures

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Organisation(en): Tofwerk AG, Schweiz

Keywords: Isomer separation, ion mobility-mass spectrometry, natural products

Einleitung
The analysis of natural products represents a major analytical challenge because of their high chemodiversity. Typically, fast LC-MS and direct infusion ultra-high resolution MS are used to investigate this type of samples but usually fail to resolve multiple isomers. Alternatively, ion mobility (IM) can be employed for separation of isomeric species without increasing cycle times and with minimal added experimental complexity. We have compared the performance of a high resolution IM coupled to TOF-MS for the analysis of natural products to a rapid LC-MS approach. Additionally, the applicability of IMS-MS for the fingerprinting of complex samples is demonstrated.

Experimenteller Teil
All IM-MS measurements were carried out on a Tofwerk IMS-TOF. The systems comprises an ESI source, a 10 cm desolvation tube, a 20 cm drift tube (both made from resistive glass) and a Tofwerk HTOF TOF-MS. Measurements were carried out in both positive and negative ion modes (applied ESI potential approximately 2kV) with IMS drift cell at pressures between 1 and 1.4 bar and temperatures between 60-100°C with nitrogen as the buffer gas. The reduced field strengths were near 2 Td.

Ergebnisse und Diskussion
Closely related isomeric flavonoids and their glycosides were analyzed by both IMS-MS and rapid UHPLC-MS. The flavonoid aglycones were all separated by ion mobility, but not by UHPLC. The glycosides were better resolved by IMS-MS but not completely separated by both methods. The combination of both methods, LC-IMS-MS, finally allowed complete separation of these isomers. The ion mobility resolving power was routinely >150, indicating that the system provides sufficient resolution for separation of isomeric natural products even in complex samples.

Therefore, IM-MS was applied to the analysis of plant extracts as well as different beverages (beer, coffee, whiskey and wine) resulting in the detection of hundreds of different features each. Many of these features originated from isomers and were only separated in the ion mobility dimension. Yet, these isomers can have a strong impact on the sample characteristics, e.g. the flavour and aroma of beverages. In the case of wine samples it is shown that the IM-MS fingerprints can be used for discriminations of grape varieties following PCA and linear discriminant analysis.

Referenzen

Ion mobility spectrometry for the study of dispersion and ionization processes in IR-MALDI

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Keywords: IR-MALDI, IMS, shadowgraphy

Einleitung

Infrared matrix-assisted laser dispersion and ionization (IR-MALDI) is a soft ionization technique which enables the direct analysis of a wide range of biologically relevant molecules in aqueous solution. IR-MALDI is based on the release of ions by liquid dispersion through the excitation of the OH-stretch vibration band of water by an IR laser pulse (λ = 2.94 µm). The dispersion process at atmospheric pressure, stemming from a laser-induced shockwave[1], has been studied by means of ion mobility (IM) spectrometry and stroboscopic shadowgraphy. Velocities for the resulting ablation plume and the TBA+ (tetrabutylammonium) ion transport have been determined at atmospheric pressure.

Experimenteller Teil

An in-house built drift tube IM spectrometer was fitted with a macro droplet IR-MALDI source consisting of a vertical metal capillary placed between its repeller and inlet electrodes. The distance between the capillary and the inlet electrode was then systematically varied to study the ions' drift velocity in the source region. At the tip of the capillary, a droplet of solution (about 1 mm in diameter) was met by the focused laser beam. The source region was kept field-free during the dispersion process and then a potential difference was applied for a short time in order to carry out a pulsed ion injection. The dispersion process was observed with a CCD camera and a stroboscopic diode.

Ergebnisse und Diskussion

The spot where the laser hit the solution droplet was chosen so that the resulting shockwave would be directed towards the spectrometer inlet electrode. Under these conditions, pulsed ion injection resulted in thesplitting up of the IM signal peaks. For simultaneous ion injection and dispersion, only single peaks were observed. As the delay between the start of the dispersion process and the ion injection was slowly increased, the peaks started to broaden and split into two: one peak corresponding to mechanical transport in the form of a pressure wave and the other, at higher drift times, to the ion injection. The position of both TBA+ peaks in the IM spectrum was reported as a function of the distance from the capillary to the inlet electrode, which gave access to the speed of both the pressure wave-induced and electrically transported ions in the source.

The IM measurements provided an insight into the velocities over a larger distance and time period. On the other hand, shadowgraphy measurements enabled the observation of the phenomenon at close range and smaller timescales. The position of the shockwave-induced ablation front was reported for the first 25 µs of the process and thus the expansion velocity in the direction of the IMS was determined for two different timescales with this method (before and after the first 5 µs).

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Mode-of-action-fingerprinting of fungicides by HS-GC-IMS

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Organisation(en): Hochschule Mannheim, Deutschland

Keywords: MVOC-fingerprinting, metabolomics, fungicide mode of action, HS-GC-IMS

Einleitung

Determination of the mode of action is an important part of the development of new agents in the agrochemical field and also the basis for the comprehension of resistances against crop protection products. Up to now, more than 40 modes of action for fungicides are known.[1] The determination of these modes of action often employs complex and time consuming metabolomic studies.[2] We are currently developing a new fast method for the determination of modes of action of fungicides based on MVOC-fingerprinting by headspace gas chromatography coupled with ion mobility spectrometry (HS-GC-IMS) on the example of the rice blast pathogen pyricularia oryzae.

Experimenteller Teil

Pyricularia oryzae was grown in liquid culture. The mycelium was separated from the culture medium, frozen in liquid nitrogen and pestled in a mortar to yield a fine powder. The powder was transferred directly into HS-GC-vials without further sample preparation. The analysis of the microbial volatile organic compounds (MVOCs) was carried out by HS-GC-IMS using an Agilent 6890N gas chromatograph (Agilent Technologies Deutschland GmbH & Co. KG, Waldbronn, Germany) coupled with an IMS-module (G.A.S. Gesellschaft für analytische Sensorsysteme mbH, Dortmund, Germany). For studying the effect of fungicides on the MVOC-fingerprint, the culture was incubated for 24 h with several fungicides and the mycelium was treated as above.

Ergebnisse und Diskussion

After optimizing the biological and technical parameters, a MVOC-fingerprint for pyricularia oryzae including more than 30 substances could be determined with good repeatability (RSD < 3 % for retention time, < 1 % for drift time and < 15 % for intensity, $n = 10$). The combination of gas chromatography with IMS as a second separation step helps to overcome issues with the separation of the complex mixture and delivers a characteristic 2D pattern of volatile metabolites. The resolution of the 2D-spectra was considerably increased compared to multi-capillary column IMS (MCC-IMS).[3] Some of the metabolites were identified by HS-GC-MS (e.g. 3-methyl-1-butanol, hexanal). First experiments on the effect of fungicides on the MVOC-fingerprint were promising, chromatograms of treated and untreated mycelium showed clearly different peak patterns. Studies targeting for a differentiation between modes of action of fungicides based on multivariate data analysis are currently underway.

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Potential of Differential Mobility Spectrometry for Mycotoxin Analysis

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Keywords: Mycotoxin, Differential mobility spectrometry

Einleitung

Mycotoxins are toxic secondary metabolites produced during all stages of crop production and food processing. HPLC-MS/MS is the method of choice to analyze food samples for mycotoxins, however in many cases strong interferences due to isobaric compounds influence detection and quantification. Differential Mobility Spectrometry (DMS) separates ions depending on their different mobility in high and low electric fields and might be a helpful tool to overcome matrix effects. The differential mobility depends - amongst others - on the identity and concentration of an organic modifier in the mobility cell. This work describes the effects of different parameters on the behavior of mycotoxins in the DMS-cell and the use of DMS as an ion filter in HPLC-DMS-MS/MS trace level analysis.

Experimenteller Teil

The effects of different modifier solvents at different concentrations on DMS parameters of six representative mycotoxins (Tenuazonic acid (TeA), deoxynivalenol (DON), aflatoxin B1 (AFB1), ochratoxin A (OTA), enniatin B1 (ENB1) and fumonisin B1 (FB1) were studied. These toxins represent polar and nonpolar compounds as well as low and high molecular weight mycotoxins. Experiments were performed on a QTRAP 5500 mass spectrometer equipped with an electrospray source and a SelexION differential mobility spectrometer. The latter was modified according to Beach et al. [1] by replacing the automatically controlled modifier solvent delivering system with an external HPLC pump. This modification allows to use a broader range of modifier concentrations in the mobility cell. The obtained results were used to implement DMS into HPLC-MS/MS analysis.

Ergebnisse und Diskussion

Some of the mycotoxins investigated are not suitable for DMS analysis; their signal intensity drops drastically when the DMS is installed between the electrospray source and the mass spectrometer. Other mycotoxins show hardly any change in signal intensity. By introducing organic modifiers in the mobility cell, the difference in DMS parameters (especially compensation voltage (CoV)) increased drastically. The broader range of CoV values facilitates the separation of analyte ions from isobaric interferences. The replacement of the integrated modifier delivery system with an external HPLC pump allowed to use lower modifier concentration than intended by the manufacturer. These low amounts of modifier yield both good signal intensity and CoV spread. HPLC-DMS-MS/MS analysis of mycotoxins in complex matrix shows far less interfering signals and improved signal-to-noise ratios compared to HPLC-MS/MS. This allows to reduce interferences during analyte identification and to lower limits of detection and quantitation.

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Quality assessment of olive oil based on volatile compound fingerprinting using HRGC-IMS analysis

Autoren: Gerhardt, Natalie; Weller, Philipp

Organisation(en): Hochschule Mannheim, Deutschland

Keywords: Authenticity assessment, Fingerprinting, Multivariate data analysis

Einleitung

The evaluation of extra virgin olive oil (EVOO) with respect to quality and authenticity has been in the focus of a plethora of studies in the past years and still poses a challenge for researches. In particular, sensitive, rapid and low-cost analytical methods are highly relevant for enforcement purposes to ensure EVOO authenticity. Non-targeted headspace analysis of volatile organic compounds (VOCs) by high resolution gas chromatography (HRGC), coupled to ion mobility spectrometry (IMS) provides an fast and effective approach for the discrimination of EVOOs of different geographical origins. This is especially due to minimal sample preparation and short run times, paired with the orthogonal separation power of retention time vs. drift time in GC-IMS.

Experimenteller Teil

All analyses were carried out on a prototype headspace high resolution GC-IMS system with a G.A.S. IMS detector. A defined amount of the olive oil sample was spiked with internal standards and then subjected to headspace analysis. The chromatographic separation was carried out on a mid-polar GC column, using nitrogen as a carrier gas. To interface the GC and an the IMS, a heated transfer line was kept at 120°C. The drift tube was operated at constant voltage of 246 V cm-1 and a temperature of 90°C under flow of nitrogenat 150 mL/min. Resulting orthogonal data (retention time x drift time) were evaluated with the LAV software by G.A.S. (Dortmund) and with custom MATLAB routines.

Ergebnisse und Diskussion

Headspace high resolution capillary gas chromatography (HS-HRGC) coupled to IMS is a very promising method for the generation of volatile composition profiles for the discrimination of extra virgin olive oils (EVOOs) of different geographical origins. Different algorithms were evaluated in MATLAB, as well as data unfolding strategies. The generated data allowed a non-targeted separation of EVOO samples with the same origin, but different qualities, as well as a tentative separation based on geographical origin. In combination with novel multivariate data analysis techniques, the data generated proved to be superior to the currently used IMS hyphenation techniques. Together with a simplified sample preparation and fast analysis times, HS-HRGC-IMS delivers a cost-efficient experimental setup.

Referenzen

Structural Investigation of Calmodulin/ Munc13 Complexes Using Ion Mobility-Mass Spectrometry

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Keywords: Ion mobility-mass spectrometry, Native mass spectrometry of protein-peptide complexes

Einleitung

The calcium-binding protein calmodulin (CaM) is a major regulator in biological processes and interacts with a large variety of targets. Among these are neuronal Munc13 proteins, which form a calcium-sensor/effector complex with CaM that controls short-term synaptic plasticity.[1] The protein CaM itself consists of two Ca2+ binding domains, which are connected by a helical linker. Compact and dumbbell-like conformations were observed for apo CaM, while earlier reported peptide-complexes almost exclusively revealed compact structures.[2,3] Here, we systematically investigate the gas-phase structure of CaM-complexes with Munc13 derived peptides using ion mobility-mass spectrometry. With regard to the function in the signaling process, it is also of interest whether the complex structure changes with different levels of free calcium.

Experimenteller Teil

Experiments were performed on a commercially available Synapt G2-S (Waters), in which the traveling wave cell was replaced by a RF-confining drift cell. This modification enables the determination of absolute collision cross sections (CCS) directly from the drift time without external calibration. The analyzed peptides share a unique CaM binding motif, but differ in single amino acids or sequence length. In addition, skeletal muscle myosin light chain kinase (skMLCK) derived peptides were analyzed to compare Ca2+ affinity and conformations of the complexes. The gas-phase structures were determined for different concentrations of free Ca2+, which should reflect the physiological conditions before and during neurotransmitter release.

Ergebnisse und Diskussion

Munc13 proteins form complexes with CaM even at submicromolar Ca2+ concentrations.[1] We here investigated the complex formation at three different calcium levels to test whether complexes form without addition of calcium (0 Ca2+) and to mimic physiological conditions for the resting (nM Ca2+) and action potential (µM Ca2+) of the synaptic process. ESI-mass spectra showed significant amounts of complex, at nanomolar calcium concentrations or even without added calcium. Furthermore, at high calcium levels quantitative binding was observed. Regardless of the free calcium concentration, the favored binding stoichiometry was a 1/1/4 complex of protein/peptide/calcium, where CaM is fully saturated with Ca2+.

The gas-phase structure of the CaM/peptide/calcium complexes was studied using ion-mobility mass spectrometry. In general, the structure of bare CaM gets more compact with increasing amount of calcium ions attached.[2] Without calcium, two conformations were observed for certain charge states. However, the intensity of the more extended species was found to decrease steadily upon addition of calcium; with four bound calcium ions only the compact conformation remained.

Ion mobility data of the investigated CaM/peptide/4 Ca2+ complexes showed only one conformational family in all observed charge states. The CCSs for these complexes were ~ 4-5 % smaller compared to bare CaM. This indicates that the so far analyzed CaM/peptide/4 Ca2+ complexes correspond to a collapsed conformation, where CaM is wrapped around the peptide. In future experiments, further peptides will be tested.

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Studying the Molecular Details of a Photoresponsive Bolaamphiphile in Gas phase and Solution

Autoren: Urner, Leonhard Hagen (1,2); Hoffmann, Waldemar (1,2); von Helden, Gert (2); Haag, Rainer (1); Pagel, Kevin (1,2)

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Keywords: azobenzene, solublility, structure-property relationship, ion mobility-mass spectrometry

Einleitung

Macroscopic properties, such as viscosity or solubility, are directly related to the structure and function of a molecule. A common route to achieve external control of those properties is based on the implementation of responsive units. One of the most explored building blocks is azobenzene, where the absorption of a photon is accompanied by 1) a change in geometry of the N=N double bond (cis or trans) and 2) the overall polarity.[1] Here we evaluate the interplay of both parameters by investigating the molecular structure of a new azobenzene-based dendritic bolaamphiphile (G1azoG1 C2), whose water-solubility was found to be strongly depending on the isomeric state of the azobenzene core.

Experimenteller Teil

To explore the origin of the photoinduced property changes of G1azoG1 C2, different condensed-phase and gas-phase techniques have been used. First G1azoG1 C2 was investigated in solution before (trans) and after (cis) irradiation at 366 nm using UV/VIS spectroscopy and reversed phase (RP) HPLC. Second, the molecular structure of isolated G1azoG1 C2 molecules was investigated in the gas phase using ion mobility-mass spectrometry (IM-MS) and gas-phase infrared spectroscopy (IR). These experiments were performed on a home-built IM-MS instrument, which was coupled to the Fritz-Haber-Institute free electron laser to enable spectroscopy experiments on size and m/z selected species.[2] Finally, molecular dynamics (MD) simulations were performed using Maestro V 10.0, Schrödinger.

Ergebnisse und Diskussion

Due to their different absorption properties, cis/trans isomers of G1azoG1 C2 can be distinguished by UV/VIS spectroscopy. Upon irradiation at 366 nm a strong blue shift of the $\pi \rightarrow \pi^*$ absorption band was observed from 356 nm (trans) to 318 nm (cis). RP-HPLC experiments, on the other hand, provided information about the overall polarity of the molecules. For G1azoG1 C2 the retention time of the cis form was found to be significantly lower than that of the trans isomer.This trend is expected, since the dipole moment of azobenzene is known to increase upon trans-to-cis isomerization. As a result, the solubility of G1azoG1 C2 increases.

Furthermore, the molecular structure of both isomers was investigated in isolation by IM-MS and gas-phase IR spectroscopy. Regardless of the isomeric state, mixtures of singly and doubly charged protonated and sodiated ions were observed by MS. For singly charged ions a single feature was observed in the IM-MS arrival time distribution, indicating that both isomers are similar in their overall shape. Further investigation on the m/z and size-selected ions through gas-phase IR spectroscopy clearly showed that the structural change almost exclusively occurs at the azobenzene core, which was further supported by MD simulations.

Taken together, the condensed phase and gas-phase experiments indicate that the substantial solubility difference between cis/trans G1azoG1 C2 is predominantly caused by the change in polarity, rather than the change in the overall geometry of the molecule.

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Posternummer: 114

Separation of isomers and conformational analysis of biomolecules by high resolution ion mobility-mass spectrometry

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Organisation(en): TOFWERK, Schweiz

Keywords: Isomer separation, conformation analysis, ion mobility-mass spectrometry, biomolecules

Einleitung
Current separation and identification techniques for isomeric substances are too often insufficiently powerful, slow or ambiguous. High resolution, low field ion mobility coupled to mass spectrometry is shown here to be a powerful, fast novel method for the analysis of isomeric biomolecules. The major advantage of IM-MS is its ability to separate isobaric and isomeric analytes by hydrodynamic “shape”, or more precisely ion-neutral collision cross sections. The measurement of these cross sections can therefore give also insight into the conformation of the analyte which can be of high interest for biomolecules where structure is strongly linked to function.

Experimenteller Teil
All measurements were carried out on a Tofwerk IMS-TOF. The systems comprises an ESI source, a 10 cm desolvation tube, a 20 cm drift tube (both made from resistive glass) and a Tofwerk HTOF TOF-MS. Measurements were carried out in both positive and negative ion modes (applied ESI potential approximately 2kV) with IMS drift cell at pressures between 1 and 1.4 bar and temperatures between 60-100°C with nitrogen as the buffer gas. The reduced field strengths were near 2 Td.

Ergebnisse und Diskussion
Here we show separation of isomeric biomolecules in a gas-tight multiplexed IMS drift cell at pressures of up to 1.4 bar. Combined with multiplexing post-processing techniques, ion mobility resolving power above 200 was obtained. Multiplexing also increases ion transmission over 200 times and S/N ratios 10 times compared to conventional pulsed mode. We demonstrate the separation of isomeric metabolites such as saccharides, sugar phosphates and amino acids as well as isomeric lipids that differ e.g. in the position or geometry (cis/trans) of the double bond.

Additionally, measurement at atmospheric pressure allows investigations into the conformations of proteins under very gentle (“native”) conditions that are thought to preserve solution-state structure better than in lower pressure systems. Data for different proteins systems that form non-covalent complexes will be presented.

Referenzen

Quality assessment of olive oil based on volatile compound fingerprinting using HRGC-IMS analysis

Autoren: Gerhardt, Natalie; Weller, Philipp

Organisation(en): Hochschule Mannheim, Deutschland

Keywords: Authenticity assessment, Fingerprinting, Multivariate data analysis

Einleitung

The evaluation of extra virgin olive oil (EVOO) with respect to quality and authenticity has been in the focus of a plethora of studies in the past years and still poses a challenge for researches. In particular, sensitive, rapid and low-cost analytical methods are highly relevant for enforcement purposes to ensure EVOO authenticity. Non-targeted headspace analysis of volatile organic compounds (VOCs) by high resolution gas chromatography (HRGC), coupled to ion mobility spectrometry (IMS) provides an fast and effective approach for the discrimination of EVOOs of different geographical origins. This is especially due to minimal sample preparation and short run times, paired with the orthogonal separation power of retention time vs. drift time in GC-IMS.

Experimenteller Teil

All analyses were carried out on a prototype headspace high resolution GC-IMS system with a G.A.S. IMS detector. A defined amount of the olive oil sample was spiked with internal standards and then subjected to headspace analysis. The chromatographic separation was carried out on a mid-polar GC column, using nitrogen as a carrier gas. To interface the GC and an the IMS, a heated transfer line was kept at 120°C. The drift tube was operated at constant voltage of 246 V cm-1 and a temperature of 90°C under flow of nitrogenat 150 mL/min. Resulting orthogonal data (retention time x drift time) were evaluated with the LAV software by G.A.S. (Dortmund) and with custom MATLAB routines.

Ergebnisse und Diskussion

Headspace high resolution capillary gas chromatography (HS-HRGC) coupled to IMS is a very promising method for the generation of volatile composition profiles for the discrimination of extra virgin olive oils (EVOOs) of different geographical origins. Different algorithms were evaluated in MATLAB, as well as data unfolding strategies. The generated data allowed a non-targeted separation of EVOO samples with the same origin, but different qualities, as well as a tentative separation based on geographical origin. In combination with novel multivariate data analysis techniques, the data generated proved to be superior to the currently used IMS hyphenation techniques. Together with a simplified sample preparation and fast analysis times, HS-HRGC-IMS delivers a cost-efficient experimental setup.

Referenzen

A targeted metabolomics pipeline for elucidating imidacloprid sublethal toxicity in the freshwater snail Lymnaea Stagnalis central nervous system

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Keywords: Metabolomics

Einleitung
Neonicotinoids pesticides received significant attention because of the possible connection to decline of honeybee colonies. Recently, more evidences suggest that the d use of this pesticide class is harmful to non-target species also. Imidacloprid is one of most employed neonicotinoids and is an environmental pollutant of concern. Imidacloprid acts as agonist on the insects nicotinic acetylcholine receptors (nAChRs) but recent reports indicate that non-target species are also affected. Therefore, the aim of this study is to elucidate imidacloprid-induced sublethal effects by a targeted metabolomics strategy in the central nervous system (CNS) of the non-target species, the freshwater snail Lymnaea stagnalis.

Experimenteller Teil
A ten days exposure to imidacloprid environmental relevant concentrations (0.1 and 1 µg/L) and higher concentrations (10 and 100 µg/L) was executed. Effects on reproduction and acetylcholine esterase (AchE) were evaluated. A metabolomics targeted approach based on HILIC-ESI-QqQ (micrOTOF, Bruker Daltonics) was employed to perform neurotransmitters profiling. The hydrophilic/hydrophobic fractions were analysed by HILIC-ESI-ToF and GC-APCI-ToF, respectively. Data analysis was performed by screening the HR-MS chromatograms for metabolomics standards using PathwayScreener (Bruker Daltonics) and the MSMLS library (IROA Technologies), consisting of more than 600 metabolites.

Ergebnisse und Diskussion
The targeted metabolomics approach based on neurotransmitters profiling and the screening of the comprehensive metabolite library enabled to reveal significant changes in hydrophobic and hydrophilic metabolites in the CNS of L. stagnalis exposed to environmental relevant concentrations of imidacloprid. Indeed, significant changes were found in many amino acids, fatty acids and neurotransmitters. Biochemical network mapping of quantitative results helped to better identify the connections between the significantly changed metabolites, and therefore the pathways potentially affected.

In this study, the involvement of different metabolic and neuronal pathways was highlighted in a non-target species. In addition, potential biomarkers of exposure have been discovered for sublethal exposure of imidacloprid in the freshwater snail L. Stagnalis. The results of this study clearly indicate that significant changes at the molecular level are induced by much lower imidacloprid concentrations than those inducing significant phenotypical effects. As a matter of fact, traditional toxicity effect parameters were compared to metabolomics and they resulted to be less effective in showing effects of imidacloprid exposure in the CNS of L. stagnalis.

Referenzen

Discrimination of the geographical origin of hazelnuts using LC-MS-based Metabolomics

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Keywords: hazelnut, geographical origin, metabolomics

Einleitung
Facing the globalization of the food market the geographical origin of food is an increasing factor for consumer buying decisions because regional products or foods of specific provenance often are associated with a higher quality and therefore of higher value. However, there are still no reliable methods for the determination of the geographical origin of foods. Mass spectrometry based metabolomics-applications are promising to be able to solve this problem. Therefore with the example of hazelnuts non-targeted UPLC-ESI-qToF-methods initially are used to detect differences in the metabolome of samples from distinct provenances. Potential marker substances with significant different concentration levels are subsequently used for down-stream processes to develop simple targeted HPLC-QqQ-Methods for routine analytics.

Experimenteller Teil
Overall 86 authentic hazelnut samples from harvest 2014 (Germany, France, Italy, Georgia, Turkey) were analyzed with UPLC-ESI-qToF (maXis; Bruker) applications. To enhance the measurable metabolite spectrum two different extraction methods were used (polar and non-polar). Four different LC-MS-methods were used for non-targeted analysis (polar using Cogent Diamond Hydride; MicroSolv column, non-polar using Accucore RP-MS; Thermo Scientific column, each in positive and negative ion mode). Calibration and feature extraction was done using DataAnalysis and ProfileAnalysis (Bruker). Multivariate analysis was carried out using Unscrambler X (Camo). The structures of 14 relevant metabolites in the non-polar/positive method were determined via fragment spectra and their exact mass. Subsequently their identities were confirmed comparing retention time and fragment spectra with commercial standard substances.

Ergebnisse und Diskussion
The PCA (principle component analysis) results for all four methods show differences in the metabolome of hazelnuts from distinct origins. The best results were achieved using the non-polar run in positive ion mode. In descending order polar/positive -> non-polar/negative -> polar/negative are suited for classification. The combination of all features from every method leads to even better results because the best predictors from each method are implemented. However, considering the intention to create simple, fast and cheap methods for routine analytics this approach is quiet unrewarding. Therefore the future procedure focuses on the most promising method, non-polar/positive. From this method 14 metabolites with significant differences between at least two countries were identified. Recalculating a PCA using only these metabolites already leads to appropriate results as well. These possible marker substances are di- and triacylglycerols, phosphatidylcholines and –ethanolamines as well as one glycidyl fatty acid ester, mainly containing either oleic acid or linoleic acid (two TAGs both). Metabolites with at least one oleic acid chain have increasing concentrations in samples from Germany over France and Italy to Turkey and Georgia. Those with linoleic acid in reverse order. Technical reproducibility is given by coefficients of variation from 2-8 % (12 QC samples) which is of great necessity for this kind of issue in order to create sufficient statistical outcome. The next step is to develop targeted HPLC-ESI-QqQ-methods for absolute quantification of these metabolites and to verify the classification efficiency. Further investigations have to proof if these results are suitable for additional samples over multiple harvest years as well and which exogenous factors lead to critical deviations. Currently 134 hazelnut samples from harvest year 2015 and several provenances as well as additional samples for other questions will be prepared for further analyses.

Referenzen

Entwicklung einer Methode zum Nachweis von Muscarin in Höheren Pilzen mittels HPLC-ESI-MS^n

Autoren: Schramm, Angela; Kemken, Dorit; Dülcks, Thomas; Spiteller, Peter

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Keywords: HPLC-ESI-MS^n, Fragmentierungsmechanismen, Muscarin, Höhere Pilze

Einleitung
Muscarin ist ein Pilztoxin, das bereits 1869 entdeckt und dessen Struktur 1957 aufgeklärt wurde. Veröffentlichungen zum Muscaringehalt Höherer Pilze sind häufig populärwissenschaftlich und oft widersprüchlich. [1, 2] Dieses kann sowohl in der Bestimmungsmethode als auch in der biologischen Variabilität der Pilze begründet sein.

Ziel der Arbeit war, eine empfindliche Methode zum Nachweis von Muscarin in Fruchtkörpern von Pilzen mittels moderner Methoden zu entwickeln, die zu einer quantitativen Methode mit möglichst geringer Bestimmungsgrenze erweitert werden kann. Da Muscarin ein permanentes Kation ohne Chromophor ist, erschien ESI-MS als Detektionsmethode in Verbindung mit HPLC im HILIC-Modus als Trennmethode geeignet.

Experimenteller Teil
Das Retentionsverhalten von Muscarin wurde an sechs unterschiedlichen Säulenmaterialien in der HPLC (Agilent 1100) untersucht. Die Detektion erfolgte durch Single Ion Monitoring mit einem ESI-Ionenfallen-MS (Bruker Esquire-LC). Anschließend wurde der lineare Bereich ermittelt und eine externe Kalibration mit einem Muscarin-Standard erstellt.

Mit der entwickelten Methode wurden vier Pilzarten exemplarisch untersucht. Die Fruchtkörper wurden mit Methanol extrahiert und diese Rohextrakte durch SPE an einem schwachen Kationenaustauscher gereinigt.

Ergebnisse und Diskussion
In der HPLC zeigte die Cyanopropyl-Phase zwar nicht die beste Manipulierbarkeit der Retention des Muscarins, erwies sich aber auf Grund des geringen Säulenblutens von den untersuchten Säulenmaterialien für die HPLC-MS-Kopplung als am besten geeignet. Eine besonders hohe Trennleistung war nicht erforderlich, da die verwendete SPE bereits sehr saubere Proben lieferte.

Ingesamt konnte eine schnelle HPLC-MS-Methode mit einem linearen Bereich von $5{,}2 \cdot 10^{-8}$ bis $1{,}3 \cdot 10^{-5}$ mol/L und einer Nachweisgrenze von 0,05 mg/kg Pilz entwickelt werden, die lediglich 200 mg eines Fruchtkörpers als Ausgangsmaterial erfordert. Die Methode erlaubt eine grobe quantitative Abschätzung des Muscaringehalts in Fruchtkörpern Höherer Pilze.

Der für einen Fliegenpilz (Amanita muscaria) ermittelte Wert entspricht den Literaturwerten. [1] Außerdem konnte Muscarin in zwei Mycena-Arten (M. pelianthina, M. pura) nachgewiesen werden, deren Muscaringehalt bisher als umstritten galt. [2]

Darüber hinaus wurden in MSn-Experimenten die Fragmentierungen von Muscarin untersucht, um die Selektivität und Empfindlichkeit der Methode gegebenenfalls erhöhen zu können. Es wurden Vorschläge für die Mechanismen erarbeitet, wobei eine zunächst unerwartete Gerüstumlagerung gefunden wurde. Eine Pilzart zeigte zusätzlich zum Muscarin ein Signal mit einer um 2 amu niedrigeren Masse. Der Verdacht, dass es sich dabei um Muscaron handeln könnte, ließ sich durch weitere MSn-Experimente erhärten.

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Fast and confident identification of drugs and their metabolites using ion trap LC-MSn analysis and a library of > 4,500 compounds

Autoren: Schneider, Birgit; Kiehne, Andrea; Meyer, Markus; Marx, Kristina; Götz, Sebastian; Baesmann, Carsten

Organisation(en): Bruker Daltonik GmbH, Deutschland

Keywords: Metabolomics

Einleitung

Comprehensive screening of urine samples in forensic toxicology and clinical research is focused on the unambiguous identification of parent drugs and their corresponding metabolites. GC-MS is currently the gold standard technology in toxicology screening due to the availability of large and well annotated spectral libraries. Here, we present the evaluation of a comprehensive and robust forensic toxicology ion trap based LC-MSn spectral library screening to detect and confirm both parent drugs and metabolites in urine as alternative or complementary technology to GC-MS.

Experimenteller Teil

Eleven urine samples were worked-up by acidhydrolysis, liquid-liquid extraction, acetylation, and analyzed after GCseparation by full scan EI-MS according to the GC-MS standard urine screeningapproach (SUSA) as published by Maurer et al. For the LC-MSn analysis, theurine samples were prepared by protein precipitation according to the LC-MSnstandard urine screening approach (SUSA). Acquired data (full scan MS, MS2 andMS3) were searched against the Toxtyper library (900 compounds) and therecently published Maurer/Wissenbach/Weber (MWW, Wiley-VCH, Weinheim, Germany,2014) LC-MSn library which contains > 4500 compound entries including 3000metabolites.

Ergebnisse und Diskussion

A combined library search approach using Toxtyper andMWW library was evaluated. In the first round spectra were searched against theToxtyper library resulting in highly reliable identification of mainly theparent drugs. In the second step non-identified compound spectra were searchedagainst the MWW library providing additional detection of metabolites andthereby increased confidence for drug identification. Most compounds could beidentified with both approaches, GC-EI-MS and LC-MSn. The GC-MS approachidentified 50 different drugs in the 11 urine samples, whereas the LC-MSn approachrevealed 60 drug identifications. Compounds such as primidone and THCcarboxylic acid were not identified by LC-MSn due to analysis in positiveionization mode only. On the other hand several hypertension drugs, antibioticsand neuroleptics such as pipamperone could be identified solely by LC-MSn. Thedetection of metabolites fortifies correct LC-MSn identifications of multipleparent compounds, e.g. butylscopolamine was confirmed by the presence of 5corresponding metabolites. Identification of drugs with fast metabolic ratesuch as propranolol (4-5 hours half-life period) is only possible throughdetection of their metabolites. Three metabolites of propanolol could beidentified via the MWW library whereas the parent compound had been alreadycompletely metabolized. The presented LC-MSn screening workflow using combinedspectral library searching of both, the Toxtyper and Maurer/Wissenbach/Weberlibrary represents a valuable tool for comprehensive and reliableidentification of toxicologically relevant compounds and their metabolites inurine, blood and other body fluid samples.

Referenzen

LCMS von Phloridzin und seinen Metaboliten im Blut und Urin der Laborratte

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Organisation(en): Max Rubner-Institut, Deutschland

Keywords: Polyphenole, Metabolismus, Ernährung

Einleitung
Phloridzin gehört zur Gruppe der Dihydrochalkon-Polyphenole und kommt vor allem in Äpfeln vor, aber auch in anderen Obstarten wie Birne oder Kirsche. Es wirkt unter anderem antioxidativ und reduziert die Glukose-Aufnahme durch Hemmung des intestinalen Glukose-Transporters SGLT1. Die systemische Bioverfügbarkeit ist relativ gering, wobei der Zuckerrest im Magen-Darm-Trakt gespalten wird und im Wesentlichen das Aglykon Phloretin aufgenommen, metabolisiert und ausgeschieden wird [1,2]. Im Zuge eines Pilotversuchs wurden Indizien für zwei unerwartete Metabolite gefunden: das intakte Glucosid Phloridzin und ein acetyliertes Phloretin.

Experimenteller Teil
Chromatographie: Acquity HSS T3, 150x2.1mm, 1.8µm (Waters); Methanol-Gradient, 0.01% Ameisensäure

Detektion: ESI-MS/MS negativ; Quantifizierung: MRM 2 Übergänge je Analyt (Acquity H-Class, Xevo TQD, Waters); matrixadaptierte Kalibration (gespiktes Humanplasma); Identifizierung: information-dependent acquisition (1290 UHPLC, Agilent; TripleTOF 5600, AB Sciex)

Tierversuch: Orale Applikation (Schlundsonde) in 0.5% Essigsäure: Phloridzin (3mg/kg, 9mg/kg) und Negativ-Kontrolle; Sektion mit Blut- und Organentnahme 30 Minuten, 60 Minuten und 24 Stunden nach Applikation (n=3 pro Gruppe); Sammelurinprobe 24 Stunden

Probenaufbereitung Plasma, Urin: mit und ohne enzymatische Spaltung Phase-II-Metabolite (Glucuronidase, Sulfatase); Proteinfällung mit Acetonitril (incl. ISTDs Narinigin, Hesperetin), Quantifizierung plus SPE (Strata-X RP, Phenomenex)

Acetylierung in vitro: Phloridzin und Phloretin in 0.5% Essigsäure, mit HCl auf 5 pH-Werte eingestellt (0.14 bis 2.0), zu verschiedenen Zeitpunkten analysiert (0 bis 7 Tage)

Ergebnisse und Diskussion
Zur Identifizierung der Metabolite wurde eine Pilotstudie mit Ratten durchgeführt. In Übereinstimmung mit einer schnellen Spaltung des Glucosids im Magen-Darm-Trakt konnten wir in Blut und Urin fast ausschließlich Phloretin-Metabolite (als Summe) sowie freies Phloretin quantifizieren. Die Konzentrationen im Blutplasma waren beim kürzesten Zeitpunkt von 30 min bereits maximal, nach 24 h waren keine Metabolite mehr nachweisbar. Die niedrigere Dosis war ausreichend für eine sichere Quantifizierung mit LC-MRM. Insbesondere bei höherer Dosierung wurden zusätzlich geringe Mengen des ungespaltenen Phloridzins gefunden.

Bei der nachfolgenden Analyse derselben Proben mittels LC-HR-MS/MS wurden, in Übereinstimmung mit der MRM-Quantifizierung, hauptsächlich das Aglykon Phloretin und dessen Metabolite identifiziert: Hauptmetabolite waren drei Glucuronide, außerdem ein Diglucuronid und zwei gemischte Glucuronid-Sulfate. Zusätzlich wurde die Identifizierung des intakten Glucosids Phloridzin per exakter Masse, Isotopenmuster und Fragmentierung in Urin und Plasma eindeutig bestätigt.

Aus einem ersten Screening ergaben sich Anhaltspunkte dass auch ein acetyliertes Produkt gebildet würde. Dies ließ sich jedoch in vivo nicht bestätigen. Da die Applikation von Phloridzin im Tierversuch in Essigsäure erfolgte, ist eine Acetylierung auch ex vivo denkbar, z.B. im Magen. Um diese Möglichkeit zu untersuchen wurden essigsaure Lösungen von Phloridzin und Phloretin nach Ansäuern mit HCl bei unterschiedlichen pH-Werten inkubiert. Dabei wurde eindeutig nachgewiesen, dass mit zunehmend saurer Lösung (pH <2) nicht nur der Zucker des Glucosids gespalten wird, sondern auch eine Acetylierung stattfindet.

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Posternummer: 121

Linking HRAM QTOF data to biology - Increasing arginine production in C. glutamicum by rational strain design and discovery metabolomics

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Keywords: Metabolomics

Einleitung
Corynebacterium glutamicum is a biotechnological workhorse for the production of amino acids and other primary metabolites. Arginine is a glutamate-derived amino acid that is used in the cosmetic and pharmaceutical industries and as a food additive. The genes for biosynthesis of arginine are organized in an operon structure (argCJBDFRGH). The suboperon argGH is transcribed from an additional promotor.

Experimenteller Teil
The genes are regulated by the repressor argR, which is activated by arginine. Additionally, the N-acetylglutamate kinase encoded by argB is feedback-regulated by arginine. Ikeda et al. [1] reported the rational construction of arginine-producing C. glutamicum strains by chromosomal deletion of argR and introduction of feedback-resistant argBfbr allels. Besides arginine, these strains accumulated significant amounts of citrulline as a by-product, indicating a bottleneck in the pathway.

Ergebnisse und Diskussion
Here we investigate three C. glutamicum arginine biosynthesis mutant strains by non-targeted HRAM LC-QTOF based Metabolomics. The novel MetaboScape software was used for automatic compound identification and mapping of detected changes to the arginine biochemical pathway map enabled interpretation of data in a biological context.

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Posternummer: 122

Metabolic Profiling of Asparagus officinalis for Discrimination of the Geographical Origin based on ultra-high resolution Mass Spectrometry

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Keywords: Metabolomics, Food Authenticity, Asparagus officinalis

Einleitung

The geographical origin of food is of high priority for German consumers, who are prepared to accept higher prices for the valuable characteristics from certain geographical areas. As a consequence, indicating the origin of goods can have significant influence on purchasing decisions and thus offers a special incentive for food fraud. Food labelling with incorrect information is a known problem these days, particularly in the asparagus production. However, there are currently no reliable methods to obtain objective evidence. Within the scope of this project, funded by BMWi/AiF/FEI, a simple and reliable LC-MS/MS-method is intended to develop for routine analysis.

Experimenteller Teil

The presented work is based on a metabolomics approach. The principle of this strategy is the assumption that the metabolom of raw material differs considerably in accordance to location and growing conditions. These differences can be identified by hypothesis-free non-targeted analysis that is able to detect as many metabolites as possible and to create a metabolic profile of asparagus samples from different growing regions (maxi fingerprint). In particular, ultra-high resolution mass spectrometry is qualified for this approach. By applying appropriate algorithms for data processing and analysis workflows, relevant marker substances are identified by using multivariate analysis. On the basis of these marker substances (mini fingerprint) a targeted method is developed by using simple triple quadrupole mass spectrometers.

Ergebnisse und Diskussion

Using authentic asparagus samples from the years 2014 and 2015, initial measurements were performed and calculated using principal component analysis (PCA). Differentiations within some German regions have been successfully detected and some first potential marker substances could be identified by ms- and ms/ms-spectra as phospholipids, monoglycerides and fatty acids. Furthermore, various exogenous influence factors, for example the determination of a representative sample size, the impact of different diameters of asparagus spears, varieties and the composition of individual stalks have been investigated since these parameters could be relevant for the selection of suitable marker substances. The results of these experiments demonstrate partly huge influences on the analysis. The next step is the validation of additional influential factors in order to identify reliable and reproducible biomarkers. Based on additional sampling and analyses, results will be checked over a longer period with regard to their consistency.

Referenzen

Profiling of reactive carbonyl compounds in biological samples by liquid chromatography-mass spectrometry

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Keywords: 7-(diethylamino) coumarin-3-carbohydrazide, lipid peroxidation; plant stress, reactive carbonyl compounds, type 2 diabetes

Einleitung

Lipid peroxidation is a complex non-enzymatic process constitutively affecting aerated tissues, known as one of the main forms of cellular oxidative damage. Under the conditions of pathology pathogenesis, and environmental stress, the rates of reactive oxygen species (ROS) production are increased, that might lead to higher concentrations of reactive carbonyl compounds (RCCs). As accumulation of RCC-derived protein modifications accompanies disease- and stress-related reactions of organisms [1, 2], enhanced production of these intermediates might be expected. Therefore, here we present LC-MS-based profiling of RCCs in the tissues of stressed plants and animals affected by type 2 diabetes mellitus (T2DM), respectively.

Experimenteller Teil

Blood plasma was obtained from 20 T2DM patients and from 20 normoglycemic individuals. The samples were derivatized with 7-(diethylamino)-coumarin-3-carbohydrazide (CHH), and lipophilic components were extracted with methyltert-butyl ether (MTBE) [3]. The same approach was applied for extraction of RCCs from the leaves of seven week old Arabidopsis thaliana plants (n = 3), grown in presence and absence of environmental stress. The extracts were analyzed by RP-HPLC coupled on-line to high resolution mass spectrometry (HR-MS) operated in the positive ion mode. The eluents A and B were water and acetonitrile, respectively, both containing 0.1 % (v/v) formic acid. After an isocratic segment, the analytes were eluted at linear gradient from 20 to 80% eluent B.

Ergebnisse und Diskussion

More than 100 plasma carbonyl CHH adducts could be effectively separated on a C4 reversed phase column and annotated by retention times, exact masses, and characteristic fragmentation patterns. The method was standardized with authentic compounds representing the major groups or RCCs – α-dicarbonyls, hydroperoxides, and unsaturated hydroxyaldehydes. The established approach demonstrated sufficient sensitivity, specificity and good reproducibility. Therefore, it was successfully applied to the profiling of the carbonyl-containing metabolome in blood plasma of T2DM patients and age- and gender-matched normoglycemic controls. Thereby, our untargeted approach relied on the label-free relative quantification, and proved to be applicable to the estimation of quantitative differences in carbonyl profiles. The same strategy was successful for plant samples, i.e. Arabidopsis thaliana leaf extracts. In both cases, quantitative changes in carbonyl profiles were characterized.

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Pyrrolizidine alkaloids in the Lolium-Festuca species complex

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Keywords: Pyrrolizidine alkaloids, grass, evolution, LC-MS

Einleitung

Pyrrolizidine alkaloids (PA) are plant specialized metabolites found in a great variety of flowering plant taxa one of which is the grass family (Poaceae) where thesinine-glycosides have been found in the widely cultivated perennial ryegrass (Lolium perenne) and the closely related talls fescue (Festuca arundinaceae) both from the grass subfamily Pooideae [1]. While much research had been conducted on pyrrolizidine alkaloids from more prominent PA-producing species not much is known about PA content, occurrence and biosynthesis in the grass family.

Experimenteller Teil

We first investigated the evolutionary origin of PA biosynthesis in grasses to determine if it coincides with the evolution of PA biosynthesis in any other PA-producing taxa. This was achieved by tracing the evolutionary origin of the pathway's key enzyme, the homospermidine synthase, using sequence-based computational phylogenetics. Beside the molecular evolution approach, we also analyzed the plants with an LC-QTOF-MS system. We screened a variety of close relatives of Lolium perenne and Festuca arundinaceae (the Lolium-Festuca species complex) for thesinine-glycosides to check how widespread PA-occurrence might be. Grass samples were analyzed with a highly standardized LC-QTOF-MS method. The analyses were conducted with a broad-band collision induced dissociation (bBCID) method to obtain MS and also MSn information for small molecules.

Ergebnisse und Diskussion

Concerning the molecular evolution aspect, we found that the HSS originated within the early radiation of the Pooideae and that many Pooideae species contain a copy of this gene, which means that these grass species meet an essential requirement of PA biosynthesis. The highly standardized LC-QTOF-MS analyses allowed us to relatively quantify the PA content in the grass samples which revealed that PAs and specifically thesinine-conjugates were only found in some representatives of the Lolium-Festuca species complex and overall PA-occurrence is very sporadic. A new discovery was that the previously disregarded species L. rigidum also contains thesinine conjugates and our analysis showed that this grass contains by far the highest concentration of these alkaloids.

The quantification experiments with the MS system revealed similar occurrences and concentrations of the PA compounds in various grasses. Moreover the concentration profile for the growing plants could also be observed. For L. rigidum the lowest PA concentration was observed after 6 week growing.

A principal component analysis was performed to evaluate the analytical stability. It could be observed that the MS results showed an intensity shift between batch 1&2 to 3&4 (each batch contained 96 samples). But within the quantification experiments these shifts were not observed, the internal standard intensities were reproducible and similar.

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Screening for Anthraquinones in Chilean Mushrooms by HPTLC - Negative Ion DESI High-Resolution Mass Spectrometry

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Keywords: HPTLC-negative ion DESI-HRMS/MS, mushrooms, anthraquinones

Einleitung

Desorption electrospray ionization mass spectrometry (DESI-MS) is a powerful ambient ionization mass spectrometric technique [1]. Its coupling with high-performance thin-layer chromatography (HPTLC) provides a simple and robust methodological approach for separation and highly-sensitive detection of natural compounds in plants and fungi [2]. Moreover, high-resolution mass spectrometry (HRMS) ensures an outstanding selectivity and specificity of this technique. This methodology can be also used in natural product research for a fingerprint analysis in crude extracts [3]. We have applied a combination of a normal phase HPTLC with DESI-HRMS for profiling of anthraquinones in Chilean Cortinarius species.

Experimenteller Teil

Crude methanolic extracts of seven Cortinarius species were investigated by thin layer chromatography using 10x10 cm Silica 60 F254 HPTLC plates. The plates were developed in a horizontal CAMAG chamber using toluene/ethyl formiate/ formic acid (10:5:3 v/v/v) as solvent system. The DESI-MS analysis was performed by an Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany) combined with a 2D-DESI-source (Omnispray System OS-3201, Prosolia Inc., Indianapolis, USA) and operated in the negative ion mode. The MS and MS2 spectra were acquired with the resolution of 30000 by scanning of individual TLC bands in the x-direction at a surface velocity of 200 µm/s. The data were evaluated using Xcalibur 2.7 SP1 software.

Ergebnisse und Diskussion

We present HPTLC-negative ion DESI-HRMS results of the analysis of complex crude extracts from seven Chilean species of the genus Cortinarius concerning the occurrence of anthraquinones. These compounds are known as potential chemotaxonomic markers in fungi [4]. Using this method physcion, emodin, dermolutein, hypericin, skyrin, 7,7'-emodinphyscion, and flavomannin C were identified by their elemental composition and partly by their characteristic fragmentation patterns in comparison with reference compounds. It should be pointed out, that the HRMS approach also allows a mass spectral distinction of isobaric ions. This was demonstrated for the detection of some investigated anthraquinones whose nominal mass is accompanied by other compounds in the crude extracts.

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Targeted metabolic profiling using high-resolution accurate mass database to identify and confirm potential biomarkers in rose and sunflower plant extracts

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Keywords: metabolomics, accurate mass, plant metabolomics, library

Einleitung

High resolution, accurate mass spectrometers are the instruments of choice for metabolomics applications or targeted metabolic profiling. They employ a high

degree of selectivity over conventional MS systems. Unbiased targeted profiling is a technique applied on QTOF instruments which allows the collection of MS and MSMS data in a single-injection. This data can be searched with a list of metabolites, from many chemical classes, pathways and species. Identification with an accurate mass MSMS library increases confidence in assignment and purity scores of unknowns from discovery experiments. A metabolite library was developed to facilitate this process. We present results that illustrate this powerful technique in a study of extracts from different lots of rose petal and sunflowers and employing accurate mass confirmation.

Experimenteller Teil

Extractions of rose petals from 3 different lots and an extraction of sunflower leaves were provided by Dr. Pfannstiel, University of Hohenheim, Stuttgart, Germany. All extracts were combined into another “pooled” lot as a control. Mass Spectrometer: SCIEX TripleTOF® 6600 System. Data were acquired in both positive and negative ion mode using both SWATHTM acquisition and TOF-IDA experiments for comparison of the MSMS quality of spectra. HPLC gradient method used an Agilent 1290 (pump, column oven, autosampler). Column: Phenomenex Kinetex® XB-C18 2.6µm, 2.1 × 100mm. Flow rate: 300 µL/min. Injection volume: 5 µL. Oven temperature: 40°C. Mobile phase: A: H_2O with 5mM NH_4OAc. B: Acetonitrile 5mM NH_4OAc.

Ergebnisse und Diskussion

Multivariate Statistical Analysis (PCA) of the different lots produced unique differences (Figure 1) between the groups of samples. Importing and processing the data sets into MasterViewTM software with the accurate mass metabolite spectrallibrary confirmed the presence and relative amounts (in relation to other groups) of flavonoids catechin, quercetin, kaempherol and phloretin as well as several endogenous organic acids. The SWATHTM acquisition sets highlighted improved coverage of fragmented precursor masses. Quality of the deconvoluted SWATHTM acquisition MSMS spectra is comparable or better than IDA-acquired product spectra providing more data points across the XIC chromatograms. The metabolite accurate mass library was developed by Gerard Hopfgartner, Emmanuel Varesio and Tobias Bruderer, University of Geneva, Switzerland.

Referenzen

Measurement of metabolic distributions of Vitamin D metabolites in human serum after chemical derivatization

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Organisation(en): Saarland University, Deutschland

Keywords: Metabolomics, LC-MS/MS, Vitamin D

Einleitung

The number and frequency of measurement of vitamin D levels in humans has significantly increased over the last decade, for assessment of public health and to support clinical diagnosis and monitoring of disease in individuals. Most current assays are limited to the main metabolite 25-hydroxyvitamin D, because of its relatively high blood levels, its long half-life and the fact that it is not feedback-controlled as other low abundant metabolites, e.g. 1,25-dihydroxyvitamin D. In this study, we report simultaneous comprehensive profiling of a wide range of vitamin D compounds using a new derivatization reagent to enhance the ionization properties.

Experimenteller Teil

Serum samples of 112 patients were obtained from an ongoing study of patients with chronic liver diseases (CLD). Samples were incubated with isotope internal standards, processed by protein precipitation, and extraction using supported liquid/liquid extraction in the 96-well format. Vitamin D compounds in the extracts were subsequently derivatized via Diels�Alder reaction using a reagent possessing a permanently-charged cation moiety. Compounds were separated via UHPLC and mass spectral data acquired in MRM mode on an electrospray ionization-quadrupole linear ion MS. Method validation was carried out in accordance with guidelines of the United States Food and Drug Administration (FDA).MRM transitions were as follows:1,25(OH)2D3,m/z 748-->689 ; 1,25(OH)2D2,m/z 761-->701; 25(OH)D3 and epi-25(OH)D3, m/z 732-->673; 25(OH)D2,m/z 744--> 685; 24,25(OH)2D3,m/z 748-->689.

Ergebnisse und Diskussion

The presentation focuses on the optimization and application of a new Diels-Alder derivatization reagent for quantitatively capturing multiple vitamin D compounds in human serum samples. The label possessed a quaternary ammonium group and derivatized compounds were thus readily transferred to the mass spectrometer as preformed ions during ESI. The data demonstrated significant improvement of the ionization efficiency of vitamin D metabolites (ca. 500´ signal improvement over underivatized compounds). Despite their ionic character, the vitamin D metabolites were successfully discriminated by UHPLC on a reversed-phase column, including vitamin D2 and D3, 25-hydroxyvitamin D2 and D3, 3-epi-25-hydroxyvitamin D3, 24,25-dihydroxyvitamin D3 and 1,25-dihdroxyvitamin D3. Limits of quantification at the lower picomolar range were obtained, readily allowing quantification of low-abundant metabolites at relevant physiological levels. Careful ESI analysis of equimolar mixtures revealed a response-leveling effect for the response factors of all investigated vitamin D compounds, due to the permanently-charged ammonium group of the derivatized molecules. This feature offered several additional advantages, including reduced ion suppression during ESI and improved quantification of those vitamin D compounds, for which no internal isotope standard was commercially available. In addition, the labeled molecules exhibited intense product ions for sensitive MRM, offering improved sensitivity and selectivity over current vitamin D assays. The new assay was used to support a clinical study of patients with chronic liver disease (CLD) undergoing vitamin D replacement therapy, with the aim of correlating measured metabolite profiles (chemotypes) with specific disease phenotypes.

Referenzen

Fragmentierungsreaktionen der Azofarben ortho- und para-Ethylrot

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Keywords: Hochauflösende Massenspektrometrie, Fragmentierungsmechanismen, Farbstoffe

Einleitung
Mit Hilfe der hochauflösenden FT-ICR Massenspektrometrie konnten wir zeigen [1], dass die doppelte Abspaltung von 44 Da aus Rhodamin B dem doppelten Verlust von C3H8 entspricht. Diese Verluste stammen zweifelsfrei aus den beiden N,N-Diethylaminoseitengruppen des Moleküls [2]. Viele weitere Untersuchungen an unterschiedlichen Systemen in unserem Arbeitskreis zeigten [3], dass dieser Alkylverlust anscheinend einem generellen Muster folgt und es sich um einen allgemeinen Fragmentierungsmechanismus handelt. Ausnahmen stellen hierbei Azofarbstoffe dar, die in ortho-Position zur Azogruppe carboxyl-substituiert sind. Hierzu wurde der Einfluss der Carboxylgruppe auf das Fragmentierungsverhalten, insbesondere der Diethylaminofunktion untersucht.

Experimenteller Teil
Alle Massenspektren wurden mit einem APEX-IV-Qe-FT-ICR-Massenspektrometer mit einem 9.4 T Magneten (Bruker Daltonik, Bremen) aufgenommen. Die Proben wurden in EtOH gelöst, in MeOH/H2O/FA verdünnt und mittels ESI-Quelle in die Gasphase gebracht. Zwei unterschiedliche Methoden wurden zur Fragmentierung der Molekülionen verwendet.

Zum einen wurden die Ionen durch Stoßaktivierung mit Argon als Stoßgas angeregt. Dies war sowohl in der ICR-Zelle als auch in einer Hexapol-Kollisionszelle im Transferbereich möglich. Zum anderen erfolgte die Aktivierung durch Bestrahlung der isolierten Ionen mit sichtbarem Licht in der ICR-Zelle. Dafür wurde ein Argon Ionen cw-Laser (Innova 70, Coherent, Santa Clara, CA, USA) im multiline Modus verwendet. Die Einstrahldauer des Lasers konnte durch einen Shutter reguliert werden. Hier wurde in einem Bereich von 0.01 bis 3.00 s gearbeitet.

Ergebnisse und Diskussion
Anders als erwartet, zeigt der Azofarbstoff ortho-Ethylrot keinen Verlust von 44 Da aus dem protonierten Molekülion. Der Verlust von Wasser führt in diesem Fall zum intensivsten Fragmentsignal. Neben diesem treten kombinierte Verluste auf, z.B. aus Wasser und einem Methylradikal oder Wasser und C3H8. Der Wasserverlust scheint hierbei die Triebkraft zu sein, um weitere Fragmentierungsreaktionenen zu initiieren. MS3 Untersuchungen haben gezeigt, dass nach dem Verlust von Wasser und des Methylradikals eine Ethylradikalabspaltung folgt. Gleiches Verhalten kann beobachtet werden, wenn das Fragment nach dem Verlust von Wasser, Stickstoff und einem Methylradikal nachaktiviert wird. Auch dieses zeigt dann den Verlust eines Ethylradikals. Beim ortho-Ethylrot kann damit gezeigt werden, dass der Abbau der Diethylaminogruppe radikalisch erfolgt, jedoch nur in Kombination mit dem Verlust von Wasser zu beobachten ist. Hinweise auf eine konzertiert ablaufende Reaktion lassen sich hier nicht finden.

Weitere Azofarbstoffe zeigen dieses Fragmentierungsmuster nicht. Befindet sich die Carboxylgruppe statt in ortho- in para-Stellung zur Azogruppe ändern sich die zu beobachtenden Fragmente komplett. Es tritt wieder der erwartete Verlust von 44 Da auf, welcher der Abspaltung von C3H8 entspricht. Ein Verlust von Wasser kann nicht beobachtet werden.

Diese großen Unterschiede im Fragmentierungsverhalten lassen sich nur durch Cyclisierungsreaktionen erklären, die durch die ortho-Stellung der Carboxylgruppe ermöglicht werden. Einen Hinweis auf diese Cyclisierung liefern die Fragmente im niedrigeren Massenbereich. Das ortho-Ethylrot zeigt auf der einen Seite ein sehr starkes Signal bei m/z 152. Dieses entspricht zwei verbundenen C6-Körpern. Auf der anderen Seite zeigt das para-Ethylrot ein sehr intensives Fragmentsignal bei m/z 134, welches nur einem C6-Körper entspricht.

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Identifikation von Limonen-Clustern im LTP-FT-ICR-MS mittels PD

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Keywords: Atmosphärenbedingungen, Cluster, LTP-FT-ICR

Einleitung

Die Bildung von Clustern aus aromatischen Verbindungen wie Benzol ist ein bekanntes Phänomen in der Massenspektrometrie. Zur Bildung dieser Komplexe werden meist Photoionisationsverfahren verwendet. Im Rahmen dieser Arbeit wurde eine Niedertemperaturplasmaquelle (LTP) für ein Fourier-Transformations-Ionenzyklotronresonanz-Massenspektro-meter konstruiert. Mittels LTP können unpolare bis polare Verbindungen geringer Molekülmasse ionisiert und deren Cluster anschließend im ICR isoliert werden. Es zeigte sich, dass unter Atmosphärenbedingungen auch nicht aromatische Verbindungen zu einer Clusterbildung neigen. Als Testsystem wurde (S)-(-)-Limonen gewählt. Die Verwendung von Photodissoziation (PD) ermöglicht eine gezielte Fragmentierung der Cluster in der ICR-Zelle und soll zur Untersuchung der Clusterbildungsprozesse genutzt werden.

Experimenteller Teil

Alle Messungen wurden mit einem 7,05 T FT-ICR Massenspektrometer der Firma Bruker Daltonik (Bremen) durchgeführt. Die Probenzufuhr erfolgte über die Gasphase. Als LTP-Quelle ist ein Eigenbau nach Nørgaard et al. [1] verwendet worden. Als Plasmagas wurde Argon (99.999 %) von Air Liquid Deutschland GmbH (Stelle) verwendet. Nach der Ionisation unter Atmosphärenbedingungen wurden die Ionen in das ICR überführt, um dort bei Bedarf isoliert zu werden. Der detaillierte Aufbau wurde 2015 von Gernert et al. [2] vorgestellt. Zur Fragmentierung mittels PD wurde ein Nd:YAG-Laser von Continuum (San Jose), der die Wellenlängen 532 nm, 355 nm sowie 266 nm bereitstellt, verwendet. Alle verwendeten Substanzen wurden von Sigma Aldrich (Taufkirchen) bezogen. Kalibriert wurde extern durch die Aufzeichnung von Limonenreferenzspektren unter Verwendung einer Elektrospray-Quelle.

Ergebnisse und Diskussion

Die durchgeführten Untersuchungen zeigten die Bildung verschiedener Cluster des Limonens. Die Hochauflösung der FT-ICR-Technik lässt eine sehr präzise Bestimmung der Analytionenzusammensetzung zu. Neben den zu erwartenden Clustern aus Kohlenstoff und Wasserstoff konnten auch Cluster nachgewiesen werden die Heteroatome wie Stickstoff oder Sauerstoff enthielten. Grund hierfür ist, dass die Ionisation unter Atmosphärenbedingungen in Gegenwart von Luft durchgeführt worden ist. Anhand der identifizierten Spezies konnten Vorschläge für sowohl die Struktur der gebildeten Spezies als auch für die Bildungsprozesse der beobachteten Cluster gemacht werden. So lassen die massenspektrometrischen Daten darauf schließen, dass innerhalb des Ionisationsprozesses nicht nur einfache Clusterprozesse stattfinden. Die Ionenverteilung und ihre Intensitäten lassen darauf schließen, dass insbesondere Prozesse, die eine formale Dehydrierung der Analytionen zur Folge haben, stattfinden müssen. Ebenfalls kann die Bildung mehrfach geladener Cluster beobachtet werden. Die Massenspektren zeigen die Existenz von Clustern der theoretischen Zusammensetzung [2M-4H+H]+, [2M-4H+2H]2+ und [2M-4H+3H]3+. Dieses Muster konnte bis zu einer Fünffachladung verifiziert werden. Mittels PD wurden erste Untersuchungen durchgeführt bei denen die beschriebenen Cluster systematisch fragmentiert wurden.

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Photodissoziations-Spektroskopie einiger Alkylhalogenide sowie Propylbenzol-Isomere

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Keywords: Metastabile Ionen, Flugzeitmassenspektrometrie, Photodissoziation

Einleitung

Bei der Untersuchung des Fragmentierungsverhaltens von Ionen können mit Hilfe der Fragmente, die aus metastabilen Vorläufern gebildet wurden, Rückschlüsse auf die Reaktionspfade der Zerfallsreaktionen gezogen werden. Es ist möglich diese metastabil gebildeten Fragmente spezifischen Vorläufern zuzuordnen. Zusätzlich kann durch die Verwendung von Lasern zur Anregung der zuvor gebildeten Ionen das Fragmentierungsverhalten bei spezifischen Anregungsenergien untersucht werden. Mittels dieser Photodissoziations-Spektroskopie können zusätzlich Einblicke in die vibronische Struktur der Ionen gewonnen werden.

Durch die Kombination von ReTOF-MS und PD-Spektroskopie können gezielt metastabil gebildete Fragmente bei verschiedenen Anregungswellenlängen des Vorläuferions untersucht werden [1,2].

Experimenteller Teil

Alle Messungen wurden an einem modifizierten ReTOF-MS durchgeführt. Die Probenzufuhr erfolgte mittels eines gepulsten Molekularstrahls mit Argon als Trägergas.

Die durch EI erzeugten Ionen wurden mit einem durchstimmbaren FL3002 Farbstofflaser (Lambda Physik), gepumpt von einem Excimerlaser (Lambda Physik), zur Dissoziation angeregt [3]. Die verwendeten Laserfarbstoffe waren hierbei Rhodamin 6G und DCM, sowohl mit als auch ohne Frequenzverdopplung. Hierbei bewegten sich die Anregungsenergien zwischen 1-2 mJ Zusätzlich wurde auch der Excimer direkt zur Anregung der Ionen genutzt. Hierfür wurde die Energie mit Strahlteilern auf etwa 5-10 mJ reduziert.

Durch die Verwendung eines ReTOF-MS war es möglich das Reflektron zur Separation der metastabil bzw. instabil gebildeten Fragmente zu nutzen.

Ergebnisse und Diskussion

Es wurden Massenspektren verschiedener Substanzen mit und ohne Anregung durch den Farbstofflaser aufgenommen und die Unterschiede der Spektren aufgezeigt. Zusätzlich wurde die freigesetzte kinetische Energie (KER) bestimmt. Durch die Anpassung der Reflektorspannung war es zusätzlich möglich, die gebildeten Fragmente anhand ihrer kinetischen Energie zu separieren und so jene aus metastabilen Zerfällen getrennt zu betrachten.

Die verwendeten Substanzen waren hierbei zunächst die in früheren Experimenten untersuchten Methyliodid, Ethyliodid sowie Propylbenzol.

Die Untersuchungen zeigen, dass es möglich ist die gebildeten Ionen gezielt zum Zerfall anzuregen und somit Spektren der Ionen aufzunehmen. Die Anzahl der zugänglichen Substanzen ist jedoch begrenzt, so war es bislang nicht möglich Spektren der höheren Homologen von Ethyliodid zu erhalten.

Ziel war es, reproduzierbare Ergebnisse zu erhalten, um anschließend verschiedene Teile des Experiments modifizieren zu können, wie z.B. den Umbau auf Laserionisation oder Änderungen am Probeneinlass. Diese Modifizierungen sollen für eine möglichst gute Kühlung der Probe sorgen, um eine Verbesserung der Spektren zu erhalten und eine größere Bandbreite an Substanzen zugänglich zu machen.

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Atmospheric pressure mass spectrometry imaging and its application in studying biological samples

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Keywords: AP-SMALDI-FT MSI, illegal drugs, xanthones

Einleitung

High-lateral and mass resolution mass spectrometry imaging (MSI) is a powerful tool with high potential for studying different types of biological samples in number of research areas and a broad range of targeted compounds classes. Commercially available atmospheric pressure scanning microprobe matrix-assisted laser desorption/ionization source AP-SMALDI (TransMit, Germany) coupled to Q-Exactive Plus (Thermo Fisher Scientific, Germany) is a system with high sensitivity and spatial resolution up to 5 µm suitable for analyzing fine biological structures. This ability of AP-SMALDI-FT MSI was tested on plant samples of Hypericum perforatum, but also on forensic hair samples or fungal mycelium.

Experimenteller Teil

In Hypericum perforatum plants we focused on distribution of xanthones in root sections. H. perforatum roots were fixed in embedding medium and cut into cross-sections with thickness of 30 µm. Dry root sections situated on the glass slide were covered by 9-aminoacridine (9-AA) and analyzed. In the second project metabolites (indigo and its precursor) formed in an interactive zone of two wood decay fungi (Schizophyllum commune and Hypholoma fasciculare)fungi1 while grown on agar plate of were imaged. Another application of AP-SMALDI-FT MSI was dedicated to study illegal substances (e.g. opioids) in human hairs. Longitudinally cut and even uncut hairs were fixed into dedicated glass slides with grooves of different width and depth and covered by α-cyano-4-hydroxycinnamic acid (CHCA) matrix.

Ergebnisse und Diskussion

Based on AP-SMALDI-FT MSI experiments with root cross-section of H. perforatum it was possible to visualize 2-D distribution of Toxyloxanthone B or 1,7-dihydroxyxanthone in sub-epidermal root-layers of the root. It is in consensus with localization of transcript and protein of enzyme benzophenone synthase responsible for xanthones biosynthesis. AP-SMALDI-FT imaging experiments which were focused on interactive zone of wood decay fungi S. commune and H. fasciculare shown 2-D distribution of indigo grains across the whole zone. Moreover, it was found that the distribution of indole as an indigo precursor was same. AP-SMALDI-FT MSI experiments proved that it is possible to analyze longitudinally cut human hairs in order to study distribution of opioids, like a heroin, codeine, tramadol or methadone as well as their metabolites. On the basis of MSI of longitudinally cut hairs the life history of individuals was studied to follow more than one and a half years of drug abuse2. Therefore we can consider designed hair cutting block as wholly suitable for these kind of MSI analysis. Moreover, ambient MSI experiments with CHCA matrix shown that drugs can be detected even from uncut hairs.

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Enhancing the Analytical Capabilities of DESI Imaging Using Ion Mobility Separation - Providing Superior Insights of Biological Samples

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Keywords: tissue imaging, MALDI, DESI, ion mobility

Einleitung
Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) coupled with ion mobility separation has demonstrated significant utility over the last decade for the separation of matrix ion species from endogenous ion species in the gas phase. Since its introduction ten years ago, desorption electrospray ionization (DESI) has been gathering momentum as a complementary MSI technique to the more traditional SIMS and MALDI-MS approaches, proving especially beneficial for the analysis of metabolites/ lipids localization in tissue. In this study, we compare and contrast DESI imaging with MALDI imaging on the same ion mobility enabled mass spectrometer, with a variety of samples. We will demonstrate that additional classes of molecules are ionized by DESI which are clearly defined using ion mobility.

Experimenteller Teil
Snap frozen tissues were sectioned at 10-15 µm onto ITO conductive or conventional glass slides and stored at -80°C until required. All experiments were carried out on a MALDI SYNAPT HDMS G2-Si mass spectrometer. When the intermediate vacuum MALDI source was in operation, a solid-state diode-pumped ND:YAG laser with a repetition rate of 1KHz was used. When the 2D DESI stage was mounted, the MALDI source was uncoupled and the electrospray inlet block was installed along with an inlet capillary. All ion mobility and mass spectrometry conditions were kept constant between both methodologies. DESI Imaging and MALDI imaging datasets were subsequently processed and visualized with the same software package where the ion mobility dimension is fully utilized and integrated.

Ergebnisse und Diskussion
Different tissue samples including mouse brain sections and human tumor sections, have been analyzed using the same mass spectrometer by both MALDI and DESI. MALDI imaging experiments required the sample preparation step of adding a matrix solution (9-aminoacridine (9AA)) sprayed in several coats using the SunCollect nebulizing spray device onto the surface of the tissue section. DESI imaging experiments require no sample preparation as desorption and ionization are initiated by charged droplets impacting directly on the surface. Therefore DESI is a simple and versatile method for obtaining localized chemical information directly from surfaces. The mass range for these experiments was typically m/z 50-1,500 and thus the compounds of interest were phospholipids, pharmaceutical compounds and endogenous metabolites. By keeping the parameters for the ion mobility and mass analyzer constant between the different techniques, the ion distribution overlap could be studied in detail. One advantage of MALDI imaging using an ion mobility enabled MS is the ability to differentiate clustered matrix peaks from the tissue derived analytes (e.g. lipids) as two distinct nested trendlines are observed in the m/z vs drift time plot. As DESI does not require a matrix compound for the ionization of molecules on the surface of tissue section, it could be expected that DESI MS spectra and 2D-plot m/z vs drift time would be cleaner. However, the DESI spectra show similar strong lipid peaks as observed in MALDI, but also intense fatty acid species were detected at the lower mass range. Closer inspection of the ion mobility dimension revealed further trendlines in the 2D-plot, corresponding to either different classes of molecules or different charge states of ions, present at much lower abundance. Investigating the origin of these ions demonstrate differences between the fundamental mechanisms of these two imaging techniques. This will be discussed with further examples presented.

Referenzen

High resolution AP-SMALDI Mass Spectrometry Imaging of Drosophila melanogaster

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Keywords: High resolution AP-SMALDI imaging, Drosophila melanogaster, Neuropeptides, Drosophila brain, insects

Einleitung

The fruit fly Drosophila melanogaster is known as a cost effective model organism, widely used to study metabolite related diseases. Among these metabolites, lipids play important roles in energy homeostasis, membrane structure and signaling. In this work high-resolution mass spectrometry imaging (MSI) was performed in order to determine the spatially resolved distribution of metabolites of D. melanogaster, with a focus on tissue-specific regional distributions. MS data were acquired with high accuracy and resolution from 20 µm thick intact cryosections of the fly. We also report an efficient method for improved preparation of tissue sections from tiny flies, challenged by hard cuticle and heterogeneous tissue types

Experimenteller Teil

Tissue sections were prepared to maintain the tissue integrity of the whole fly. Samples were stored at -80 ºC until sectioning. All imaging experiments were accomplished by using an atmospheric pressure scanning microprobe matrix assisted laser desorption/ionization imaging ion source (AP-SMALDI10®, TransMIT GmbH, Giessen, Germany), coupled to a Fourier transform orbital trapping mass spectrometer (Q ExactiveTM, Thermo Fisher Scientific GmbH, Bremen, Germany). For soft ioniztion, 2,5 dihydroxybenzoic acid (DHB) or paranitroaniline (pNA) were homogeneously deposited as an ultrafine matrix spray by using a dedicated matrix preparation unit (“SMALDI Prep”, TransMIT GmbH, Giessen, Germany)

Ergebnisse und Diskussion

We identified and characterized the anatomical distribution of a total of 97 lipids, with 62 of them identified as glycerophospholipids and sphingolipids and 35 of them as glycerolipids, in several biological replicates of the fly, with consistent anatomical distributions within the lateral pixel resolution of 5-10 µm when using 2,5-dihydroxybenzoic acid (DHB) as a matrix in positive-ion mode. Additionally, we used paranitroaniline (pNA) as a matrix for both positive- and negative-ion mode, resulting in the identification of 89 deprotonated lipids in negative-ion mode. Among them, 48 were identified in both positive- (protonated) and negative-ion (deprotonated) mode within a mass accuracy of ±3 ppm with similar spatial characteristics. All ion images also showed regional separation of lipids according to their principal locations in head, gut and whole body of the fly. Especially, the molecular topography of Drosophila brain metabolites, including lipids, small metabolites, and neuropeptides, may provide the possibility to study neurodegenerative diseases. Therefore, we additionally mapped the spatial distribution of neuropeptides in coronal Drosophila brain sections. Furthermore, several lipid-based male- and female-specific sex pheromones were identified, differentiated and characterized according to their typical spatial distribution, both in mated adult and in virgin flies. Our instrumentation provided high mass resolution and mass accuracy, combined with on-tissue MS/MS acquisition, providing precise and detailed information from internal-organ-specific tissues. By using DHB, our approach allowed the identification of protonated, sodiated and potassiated lipid species within a mass accuracy of ±1 ppm and lipid-based pheromones within ±3 ppm, with a lateral pixel resolution of 5 to 15 µm at a high detection sensitivity

Referenzen

In-depth identification of protein images by combining high mass resolution MALDI FTICR Imaging and high performance qTOF nLC-MS/MS

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Keywords: MALDI FTICR Imaging

Einleitung

In discovery-mode MALDI imaging one major challenge is to identify putative marker proteins. On-tissue enzymatic digestion is one strategy which allows for finding putative marker peptides and then performing MS/MS to identify the originating protein. Carrying out experiments like this directly from tissue can be quite limiting and presented here are results using an alternate strategy in which tryptic peptides are imaged using high resolution FT-ICR and matched to peptides extracted from an identically digested serial section and identified by LC-MS/MS using a qTOF.

Experimenteller Teil

Serial sections of fresh-frozen rat testes were thaw mounted onto ITO slides, dried, and washed. Slides were then dried in vacuum for 15 minutes before applying the enzyme. Solutions of HCCA and trypsin were applied using a TM-Sprayer. For digestion, slides were incubated at 37 degrees for one hour in a closed humid environment. Prior to matrix application, peptides from one section were extracted by depositing 20ul of 0.1% formic acid onto the section, gently stirred, and then transferring into a tube. Matrix was applied to the second section and ion images were acquired using FT-ICR. Proteomic analysis of the extracted peptides was done using a RSLCnano/pepmap column coupled to an impact II qTOF with Captive spray nanobooster source.

Ergebnisse und Diskussion

Sections of rat testes present a dense microtubule architecture containing a highly localized protein environment. For MALDI imaging, the standard TM-Sprayer conditions were found to yield more than one thousand tryptic peptides detected in the MALDI spectra with the ICR resolving up to 8 peaks at each nominal m/z. Using the smallest software setting for the laser focus yields ion images which exhibit spatial resolution on the order of 25 um/pixel.

Proteomics analysis of the extract resulted in 300 proteins identified using a gradient of 80 minutes and the Instant Expertise Identified algorithm. Peptide results were exported from ProteinScape to Excel format and then manually matched with corresponding ion images according to m/z using an acceptance window of 5ppm. In this manner, nearly half of the identified proteins, 137, could be putatively assigned to ion images.

Referenzen

Investigating the uptake of mercury species in Drosophila melanogaster by quantitative elemental bioimaging

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Keywords: Mercury species, elemental bioimaging, Drosophila melanogaster

Einleitung

The toxicity of the heavy metal mercury is significantly depending on its chemical species which can be categorized into elemental (Hg0), inorganic (Hg2+, Hg22+) and organic (e.g., MeHg+, EtHg+) mercury. Although the toxicology is highly investigated, only little is known about the distribution and metabolism of mercury species in biological organisms. The high affinity of mercury to sulfhydryl groups e.g. in the amino acid cysteine is well understood. Moreover, the ability of methylmercury and ethylmercury to cross the blood-brain barrier could be shown when bound to particular amino acids. In this study, the model organism Drosophila melanogaster was selected to examine the uptake of mercury species. Quantitative, spatially resolved information could be obtained using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS).[1]

Experimenteller Teil

Drosophila melanogaster wildtype were fed with the mercury species mercury(II) chloride, methylmercury chloride and thimerosal. Cryosections of the gelatin embedded entire larvae and the cerebral areas of larvae and adult flies were prepared after infiltration with sucrose solution to cover the nonpolar surface. The mercury distribution within the larvae and adult flies was examined using a 213 nm Nd:YAG laser coupled to a quadrupole based ICP-MS. Next to the dry aerosol of the ablated material, a nebulized bismuth solution was introduced into the plasma to monitor and enhance plasma stability. Cryosections were ablated line-by-line with a laser diameter of 10 µm. Quantification was performed with homemade matrix-matched standards based on gelatin using meso-2,3-dimercaptosuccinic acid (DMSA) for mercury complexation.

Ergebnisse und Diskussion

A quantitative method for the spatially resolved determination of mercury species in the model organism Drosophila melanogaster was developed and applied to larvae and adult flies. For quantification of mercury, matrix-matched standards based on gelatin were prepared. Strong evaporation issues and an inhomogeneous distribution of mercury within the standards, due to interactions with cysteine containing proteins of the gelatin, were successfully handled by complexation with meso-2,3-dimercaptosuccinic acid (DMSA). The quantitative mercury distribution in Drosophila melanogaster was investigated for the three species mercury(II) chloride, methylmercury chloride, and thimerosal after oral uptake. Next to the analysis of entire Drosophila melanogaster, particular focus was directed to the cerebral areas of larvae and adult flies. In case of mercury(II) chloride, no mercury was detected in the cerebral region, whereas both organic species were able to cross the blood-brain barrier correlating with the significantly higher toxicity of organic mercury species compared to inorganic ones. The mercury level in the brain exceeded the fed concentration indicating a mercury enrichment. This was approximately three times higher for methylmercury chloride than for thimerosal.

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Laser ablation-atmospheric pressure chemical ionisation-mass spectrometry for the molecular analysis of liquid crystal displays (LCDs)

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Keywords: Laser ablation, liquid crystals, atmospheric pressure chemical ionisation

Einleitung

Many flat panel displays in electronic devices e.g., televisions, are based on the liquid crystal display (LCD) technology. Small organic molecules (liquid crystals (LCs)) are important for the image buildup. A mixture between ten and twenty LCs form the basis of the liquid crystal layer in a LCD.[1] For the correct function of a LCD, the distribution of the organic molecules within the layer has to be homogeneous. Laser ablation coupled to atmospheric pressure chemical ionisation-mass spectrometry (LA-APCI-MS) can be used to obtain lateral information about the previously mentioned layer in LCDs.

Experimenteller Teil

Different types of liquid crystals were analysed with LA-APCI-MS. Solutions of these compounds were individually spotted on a thin layer chromatography plate. The resulting spots were linewise ablated with a 213 nm Nd:YAG laser. The ablated material was transported by a nitrogen gas stream to the APCI interface for ionisation. The resulting ions were detected with an orbitrap mass spectrometer. For the direct analysis of the liquid crystal layer a commercial LCD was used. Initially, the two plates of glass, which embedded the layer, were separated and the plate without colour filter was used for ablation.

Ergebnisse und Diskussion

The correct function of LCDs requires a homogeneous arrangement of LCs within the layer. The analysis with chromatographic methods e.g., HPLC/MS or GC/MS, generates information about contained analytes in the mixture, but lateral information is consequently lost, since the mixture has to be leached from the display before analysis. The surface study of the layer requires imaging techniques such as LA-APCI-MS.

A method for the investigation of the liquid crystal layer of a LCD is demonstrated. Therefore, single substances are examined with LA-APCI-MS with regard to their ionisation behaviour and fragmentations. The exact m/z value allows the identification of the compounds and their fragmentation products. Subsequently, the method is applied to a liquid crystal layer. Most of the previously detected substances can be simultaneously identified within the layer. LA-APCI-MS as a molecular imaging technique offers the possibility to generate lateral information about organic molecules in LCDs without sample pre-treatment. Furthermore, this method can be used to determine the distribution of liquid crystals within defect areas and potentially identify the causes for failure.

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Laser Mass Spectrometry Imaging of Cuticular Lipids from Insects Using Etched Silver Substrates

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Keywords: Laser mass spectrometry, Silver substrates, Saturated hydrocarbons, Cuticular lipids, MS imaging

Einleitung

UV-LDI mass spectrometry can be used to analyze cuticular lipids (including unsaturated hydrocarbons, alcohols, acetates, fatty acids, and triacylglycerols) directly from insect cuticles [1, 2]. However, due to the lack of functional groups, saturated long-chain hydrocarbons (HC) are not detected by UV-LDI-MS. Previous studies showed that long-chain alkanes can be analyzed by UV-LDI-MS if AgNO3 or Ag nanoparticles are added to cuticular extracts, giving rise to [M + Ag]+ ions [3]. Here we present a modified approach in which etched silver substrates are used, instead. We show that this enables imaging of flat insect surfaces (such as wings) after blotting.

Experimenteller Teil

Silver substrates were etched using nitric acid at 50 °C until the surface appeared gray-ish due to enhanced light scattering. SEM images revealed pore sizes of the surface in the 10 µm-range. For analysis of cuticular extracts or lipid standards, 0.1 µL were spotted. For imaging experiments, investigator's finger or insect wings were pressed onto the substrate. MS experiments were performed with a modified MALDI Synapt G2-S mass spectrometer (Waters) and a ~5-10 µm-wide focal spot of a N2 laser beam [4]. The buffer gas pressure in the ion source was set to 0.7 mbar. Additional MS experiments were performed with a high vacuum (pion source ~10-7 mbar) (Autoflex speed; Bruker Daltonik) and an atmospheric pressure instrument (iMScope TRIO; Shimadzu).

Ergebnisse und Diskussion

Using the Synapt, LODs were determined with standards to fall typically into the 100 fmol range, with the exact values depending on chemical composition and degree of unsaturation. All compounds were predominantly detected as [M + Ag]+ species. A drawback is the detection of ion doublets owing to the isotopic distribution of silver (107Ag/109Ag). Compared to weak [M + K]+ or [M + Na]+ adducts as typically detected by UV-LDI-MS, [HC + Ag]+ adducts of polyenes and oxygen-containing lipids are however stronger bound. This relaxes the requirements on collisional cooling in the ion source and, at least for less volatile lipids, supports their detection with axial-TOF instruments. By pressing a HC-coated TEM sample grid with defined structure onto the Ag surface, followed by MS imaging at 15 µm step size, the lateral resolution of the MSI analysis could be estimated to be in the range of at least 25 µm (the width of the TEM grid bars). The MS images of the fingerprint showed the lateral distribution of several squamous components (e.g. Cholesterol and Squalene). MS images recorded after blotting a hind wing of a male Bicyclus anynana butterfly showed a rich lipid composition. The two male sex pheromones hexadecanal (C16:0-ald) and 6-10-14-trime-15-2-ol (C18:0-OH) [5] showed a lateral distribution reflecting their passive transport from the androconia gland via wing hairs. The described technology can enable MS imaging of a wide range of very hydrophobic to more polar analytes, including saturated and unsaturated hydrocarbons, alcohols, free fatty acids, wax esters, sterols and triacylglycerols. Using the blotting approach, the MS analysis is possible from samples that are not amenable to direct UV-LDI-MS (e.g., because lacking a sufficient optical absorption at the laser wavelength) or to MALDI (e.g., because of a lack of functional groups for ionization).

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Posternummer: 138

Lipid profiling of mouse lung using AP-SMALDI mass spectrometry imaging

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Keywords: MALDI mass spectrometry imaging, lipids, lung, spatial distribution, Mirion.

Einleitung

Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) is a prominent analytical tool to identify and understand the spatial distribution of biomolecules such as lipids, proteins and metabolites in biological tissue. Being a label free method and it can detect hundreds of molecules from direct tissue in a single experiment, MALDI MSI has received attention over other mass spectrometry and conventional imaging methods.

Lipids are the biomolecules which plays an important role in structural component of the cell membrane and various cellular metabolism. In lungs, lipids act as a signalling molecules for inflammation. In this study, MALDI MSI technique was used to study the complete lipid profile of mouse lung tissue.

Experimenteller Teil

Mouse lung tissue sections of 12µm thickness were obtained using a cryomicrotome at -20 oC. Matrix was sprayed uniformly onto the tissue sections, using a pneumatic sprayer ("SMALDIPrep", TransMIT GmbH, Giessen, Germany).

An atmospheric-pressure scanning microprobe MALDI mass spectrometry imaging ion source ("AP-SMALDI10", TransMIT GmbH, Giessen, Germany), coupled to a Fourier transform orbital trapping mass spectrometer (Q Exactive, Thermo Scientific GmbH, Bremen, Germany) was used for imaging experiments1. The lung tissue sections were analysed with a high resolution in mass (140,000@ m/z 200) and space (10 µm per pixel). Characteristic matrix peaks were used as lock mass signals for internal calibration. Data was processed and images were generated using MS imaging software "Mirion" and "MSiReader".

Ergebnisse und Diskussion

To obtain optimal staining results for visualization of distinct lipid classes with no background staining, mouse lung cryosections were with the thickness of 12µm were mounted on a glass slide without using OCT embedding material. Optical images of the lung tissue sections were taken before mass spectrometric analysis. Small and homogeneously sprayed matrix crystals were obtained on tissue sections by using SMALDIPrep unit.

Mass spectrometry imaging data of lung tissue sections were acquired in both positive and negative mode using AP-SMALDI10 / Q Exactive platform in high-resolution mode in the mass range of 250-1000 m/z. Mass images were generated by processing the raw data using the image processing software system Mirion with a bin width of 0.01 (Δ m/z). Resulting mass values were searched in Metlin database for lipid annotation in both ionization modes. A list of lipids (mainly surfactant lipids, fatty acids, and phospholipids) was obtained with mass accuracy below 1 ppm root mean square error (RMSE) and this list was used for further data analysis of mouse lung tissue using MSiReader software. Mass spectrometry imaging experiments will be helpful to understand the complete profile and spatial distribution of mouse lung lipids.

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MALDI imaging of on-tissue digests at high spatial resolution

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Keywords: On-tissue reactions, high spatial resolution

Einleitung

MALDI imaging resolution of on-tissue enzymatic reaction products is limited by analyte delocalization induced during enzyme deposition and humidified reaction conditions. Resolution is often affected further when matrix is deposited under conditions conducive to analyte extraction and co-crystallization. Aerosols remain the most reliable method of depositing fine drops of enzyme or matrix onto tissue and the size of the droplets directly affect achievable spatial resolution. Here, is an examination of different preparation conditions that have been carried out to optimize digestion rates while maintaining laser-limited spatial resolutions.

Experimenteller Teil

Sections fresh-frozen rat testes were thaw mounted onto ITO slides, dried, and washed. Slides were then placed under vacuum for 15 minutes prior to enzyme application. Solutions of matrix (HCCA, 5mg/ml) and trypsin (80ng/ul) were appliedusing a TM-Sprayer. Trypsin was sprayed at 7.5ul/min and matrix at 100ul/min. Standard sprayer settings were 750mm/min, 8 passes with alternating tracks, and 30 degrees. Slides were incubated at 37 degrees for one hour in a closed humid environment prior to applying matrix. Various amounts of trypsin were applied and two different incubation times were evaluated. Ion images were acquiredusing FT-ICR and MALDI-TOF at spatial dimensions of 10 and 25 um/pixel.

Ergebnisse und Diskussion

Sections of rat testes, with their tightly packed network of microtubules and interstices, provide an ideal model for evaluating high resolution ion images. Standard sprayer conditions yielded significant tryptic peptides from both solarix XR and ultraflextreme with laser focus at the minimum setting. After imaging, matrix was removed from the slides allowing the sections to be stained with H&E. Referencing architectural features visible in both the histology and ion images demonstrates that the ion images exhibit resolutions comparable to the focused laser size and do not appear to be degraded by migration during the application of enzyme or matrix or during the incubation.

Digestion conditions were varied in an attempt to determine if enzyme kinetics could be improved and if so, these conditions foster peptide migration, limiting spatial resolution. To identically prepared sections, trypsin was deposited at 200, 400 and 600 ng/cm2, as determined using a formula provided by HTX, by passing the sprayer over the tissue more times. After incubating for 2 hours each section was measured and extracted ion images from these sections exhibit similar ion intensities and spatial resolutions indicating a) the higher amounts of trypsin don't increase the kinetics and b) the increased amount of aerosol deposited doesn't promote analyte migration. In a subsequent experiment, two sections were coated with 200ng/cm2 trypsin and incubated for 2 hrs and 4 hours, respectively. Extracted ion images of tryptic peptides did not exhibit greater intensity at the longer incubation time.

Referenzen

MALDI-MS Imaging with a Synapt Mass Spectrometer: Improving the Performance Characteristics by Buffer Gas Control, Laser Beam Shaping and Postionization

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Keywords: Collisional cooling, laser beam shaping, postionization

Einleitung

MALDI Synapt mass spectrometers (Waters) are widely employed for MS imaging. A particular feature of these QTOF-type instruments is the implementation of a fine-vacuum ion source (by default operated with an N2 buffer gas pressure in the low 0.1 mbar range). The default lateral resolution of the current G2-S series is about 50 µm (without oversampling). Here, we demonstrate that this value can be lowered to <10 µm by use of laser beam shaping [1,2] and that the detection of labile molecules can be enhanced by raising the buffer gas pressure to ≥1mbar for improved collisional cooling [3]. The increased pressure also forms a key prerequisite for the successful implementation of our recently introduced postionization (PI) method, named MALDI-2 [2].

Experimenteller Teil

By use of a wavelength-tunable laser, secondary MALDI ionization processes are initiated in the buffer gas environment. This boosts the ion yields of numerous phospho- and glycolipids as well as that of cholesterol, fat-soluble vitamins and saccharides. The ion source of the Synapt G2-S was modified to enable operation at elevated buffer gas pressure (N2) of up to 3 mbar at the sample region. The beams of the default Nd:YAG-laser (Flare, InnoLas; λ=355 nm) or that of an N2 desorption laser (MNL 100-LD, LTB Berlin; λ=337 nm) were first expanded using a telescope and then spatially mode-filtered.

Ergebnisse und Diskussion

This together with placing the final focusing lens (f=60 mm) inside the ion source reduced the effective laser spot diameter (area of visible material ablation) to ~5 µm. An extra port in the vacuum housing served for coupling the beam of an optical-parametric oscillator (OPO) laser (versaScan, GWU Lasertechnik). The focal beam waist of the PI laser was about 100 µm wide and centered about 500 µm above the desorption site. Pulse sequences between desorption and PI laser (frep=20 Hz) were adjusted using a custom-made delay generator and controlled via photodiodes. Only the N2-laser was used in the PI experiments.

Using laser beam shaping the lateral resolution of conventional MALDI-MS imaging experiments could be increased to 5-10 µm. By making use of the new MALDI-2 option the ion yields for numerous metabolites and lipids desorbed from plant and animal tissue could be improved by up to 2 orders of magnitude. Ion suppression effects as notorious in conventional MALDI (e.g., caused in the positive in mode by the presence of abundant phosphatidylcholines) were substantially reduced. Both features greatly facilitate obtaining comprehensive ion profiles at 5-10 µm resolution. Next to describing the technical set-up, exemplary results obtained with a 2,5-dihydroxybenzoic acid (DHB) and a norharmane matrix from animal and plant tissue will be presented.

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Overview of software tools for processing of MSI data

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Keywords: Mass spectrometry imaging, MSI data visualization tools, imzML, MSI data repository

Einleitung

Mass spectrometry imaging (MSI) is used to analyze distribution of compounds over the surface of biological sample. MSI data is a complex dataset comprising of spatial information correlated with multiple ionizable compounds present at each pixel. MS instrument vendors and many bio-informatics groups have come up with various software to analyze MSI data. However, the main difficulty for biologists or clinicians is to analyze, merge and compare of data from different instruments on the same platform. Since 2009, a common data format known as imzML has been developed (www.imzml.org).1 imzML, being the vendor neutral data format, facilitates the flexible sharing of MSI data and its visualization into various available visualization tools without restriction to proprietary vendor software

Experimenteller Teil

imzML data format forms the base for this study. We used a imzML format MSI dataset published and made publicly available by Römpp et al (2010) via ProteomeXchange repository (accession number PXD001283).2 This data is in processed mode where a m/z array is stored for each spectrum separately. We also used another MSI TOF-dataset in continuous mode where an intensity value is stored for each m/z. We visualized above data into various commercial (MALDIVision, Spectviewer, Mirion) or freely available (DataCubeExplorer, BioMap) or open-source software (MSiReader, OmniSpect, Cardinal) to assess their suitability and ease to the end user.

Ergebnisse und Diskussion

The concept of imzML is actively supported by major vendors of MS imaging instrumentation (Thermo Fisher Scientific, Waters Corporation, Bruker Daltonik and Shimadzu). Various MS vendor software now offer support for conversion of vendor specific MSI data format into imzML. (eg, Waters' High Definition Imaging (HDI), Bruker flexImaging, Thermo ImageQuest etc). Other software packages also have been implemented for this purpose (eg Thermo Raw to imzML convertor, ToImzmlModule, mzML convertor etc). imzML data is easily stored, retrieved and analyzed with various available visualization tools such as DataCubeExplorer, BioMap, MSiReader, OmniSpect, Spectviewer, MALDIVision, Mirion, and Cardinal. These software have many common but few software specific processing functionalities like the mode of data they could analyze (processed vs continuous), data representation, color scales, ROI selection capabilities, overlay & co-registration of images, smallest allowed m/z bin width etc, which render them unique. Here, we evaluated and compared graphical representations obtained in various visualization tools. We describe strengths and weaknesses of available visualization tools.

MSI field is slowly maturing and progressing towards unified standards. Common data format like imzML, online storage repository for MSI and freely available imzML visualization tools will pave the way for evaluation and comparison of MSI data generated from different laboratories. This is exemplified in a recent study comprising of imaging analysis of mouse brain sections conducted in five laboratories in Europe and USA.3 Such model of multicenter studies in combination with reporting guidelines, common data format (imzML) and access to public repositories will facilitate the realization of multi-laboratory collaborative research and also build reliability and transparency in MSI field.

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Quantitative bioimaging of platinum via on-line isotopic dilution-laser ablation-inductively coupled plasma-mass spectrometry (ID-LA-ICP-MS)

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Keywords: Cisplatin, elemental bioimaging, on-line isotopic dilution, laser ablation, inductively coupled plasma mass spectrometry

Einleitung

Platinum-based anti-cancer drugs play an important role in modern chemotherapy. Cisplatin [cis-diamminedichloroplatinum(II)] is the most commonly applied compound due to its unmatched curing chances for certain types of cancer like testicular, ovarian, bladder and lung cancer. [1]

The visualization of elemental distributions in biological tissues may provide valuable information about biological and medical correlations. LA-ICP-MS is one of the most promising analytical methods because it features high sensitivity, spatially resolved and multi-elemental analysis and enables quantification over a broad concentration range. [2]

In this study, a novel method based on on-line ID-LA-ICP-MS for bioimaging is presented. Therefore, platinum was chosen as the element of interest and the method was applied to rat kidney samples from Cisplatin kidney perfusion experiments.

Experimenteller Teil

Rat kidney samples from Cisplatin perfusion experiments were embedded in Technovit. Platinum Technovit standards and samples were cut in 5 µm slices and ablated with a 213 nm Nd:YAG laser. The generated particles were transported via a helium gas flow into the quadrupole-based ICP-MS. The 194Pt isotopically enriched spike was added on-line after the laser ablation cell using an Apex Q dry aerosol generator.

Platinum quantification was performed by both external calibration and the developed isotopic dilution approach in a line-by-line scan using a laser spot diameter of 50 µm.

Ergebnisse und Diskussion

An instrumental setup for on-line ID-LA-ICP-MS was designed regarding the introduction of the 194Pt isotopically enriched spike and an appropiate aerosol mixing for achieving the isotopic equilibrium. The final instrumental setup included a post-cell introduction of the 194Pt enriched spike via the Apex Q and no subsequent elongation of the pathway for aerosol mixing.

Furthermore, the conceptional approach of the platinum on-line ID-LA-ICP-MS bioimaging was developed including the spike mass flow determination via reversed IDA and the calculation of the final concentrations for each pixel of the recorded bioimage.

As proof of principle, several platinum bioimages of rat kidney samples from Cisplatin kidney perfusion experiments were analyzed. The comparison of the external calibration and the IDA method shows high accordance in all cases, concluding the general comparability and applicability of the developed method. In addition, the results of the real sample analysis showed that the spatially resolved analysis via on-line ID-LA-ICP-MS is practicable and similar results to previous studies were obtained.

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The Effect of Laser Spot Size and Beam Intensity Profile in MALDI-MS Imaging and Steps towards Improved Oversampling

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Keywords: MALDI-Imaging, Laser Beam Profile, Oversampling

Einleitung

The lateral resolution in MALDI-MS Imaging is largely defined by the dimensions of the focal laser spot. Flat-top profiles, that have the advantage of providing a uniform deposition of the laser energy, can be obtained by beam shaping. Particularly interesting are flat-top profiles with square cross-sections because they allow for most controlled material ablation. Here we studied the material ablation (recorded by a photoacoustic method [1]) and ion generation as a function of spot size and type of beam profile (Gaussian vs. flat-top) for conditions of MALDI-MSI. Using a QSTAR instrument and a square laser profile we recorded high-quality MS images from mouse brain tissue in oversampling mode at about 20 µm resolution.

Experimenteller Teil

A fundamental beam shaper (FBS, TOPAG) was used to generate a square flat-top profile. The generated beam was split and imaged onto the MALDI sample plate and a beam profiler chip using a telescope with different magnifications. In this way, spots with cross sections between 20 – 200 µm were produced. A Nd:YAG laser (l=355 nm; 300 Hz; Diodescope, Bioptic Lasersysteme) was used as excitation laser. MS data were recorded with a QSTAR pulsar-i mass spectrometer. For most MS imaging experiments continuous-raster-mode was used with the slowest possible stage movement, leading to a 20 µm pixel size in raster-direction. In the other direction 20 µm pixel (if realized) were achieved with different spot sizes by oversampling.

Ergebnisse und Diskussion

For a first fundamental study, previously reported results on the dependence of the ion signal on laser spot size and fluences [2, 3, 4] were revisited with the given setups for both Gaussian and square flat-top beam profiles. The dataset was extended with complementary measurements of the material ablation via a photo-acoustic setup [1]. The combination of data sets of both ion intensity and ablation measurements enable to determine optimal conditions for a maximized ion yield (ions per material ablated). The extensive data can also be used to shed some light onto effects causing performance differences of MALDI at different laser spot sizes that are currently not comprehended.

Furthermore, in a more application-centered approach, imaging experiments with mouse brain were performed using laser spot sizes in the range from 20 – 200 µm and both beam profiles. Especially oversampling experiments with step sizes of 20 µm were used to compare ion signals and image resolution under different excitation conditions regarding beam size and shape. Since the QStar can be run in a raster-imaging mode, full mouse brain images with a lateral resolution of 20 µm can be recorded in about six hours, resulting in contrast-rich MS images.

Overall, the differences between a Gaussian and a flat-top profile seem to be more pronounced with larger spot sizes and are less visible with spot sizes smaller than 50 µm.

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Posternummer: 144

Viscous Ionic Liquid Matrices for MALDI-MS Imaging of Brain Lipids with High Lateral Resolution

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Keywords: MALDI MSI, ionic liquid matrices, mouse brain

Einleitung

Analytical sensitivity and lateral resolution are key factors in MALDI-MS imaging and are critically determined by the physico-chemical properties of the matrix-solvent system and the morphology of the coating. A few studies have previously reported the use of viscous room temperature ionic liquid (RTIL) matrices for MALDI-MSI of phospho- and glycolipids [1, 2]. Step sizes of 50 and 75 µm were used in those works, which limited predictions about a possible analyte diffusion preventing an even higher resolution. Here we synthesized a total of 180 RTILs and identified 7 as particular suited for MALDI-MSI of lipids (6 of which were not previously described for MALDI). Using these 7 compounds we analyzed mouse brain lipids at a step size of 15µm.

Experimenteller Teil

RTILs were synthesized using a proton exchange reaction and (typically) using a classical UV-absorbing MALDI matrix as the acidic component. To screen the principle suitability of the generated RTILs (i.e., the subgroup which provided the desired viscous gel-like morphologies and a sufficient optical absorptivity), a modified QStar-type mass spectrometer (AB Sciex) was used. A modified MALDI Synapt G2-S mass spectrometer (Waters) served for high-resolving MSI experiments of the 7 selected RTILs. By use of laser beam shaping [3], an effective focal spot size (area of ablation) of about 10µm in diameter was achieved with the employed N2-laser. Matrix coating were produced with an airbrush sprayer and chloroform/methanol as solvent system. All mass spectra were recorded in the positive ion mode.

Ergebnisse und Diskussion

Among the 7 most suitable RTILs for the MSI analysis were 4 that are based on either 2,5-dihydroxy benzoic acid (DHB) or a-cyano-4-hydroxycinnamic acid (CHCA) as cation. Although DHB- and CHCA-based RTILs were used before for MALDI-MS(I), 3 out of 4 of the used counter ions were not described previously. Next to DHB and CHCA, in particular two RTILs with ferulic acid and coumaric acid as cations provided a notable performance. All selected RTILs provided a high analytical sensitivity (similar to tissue coatings with crystalline DHB matrix). However, the obtained signal intensities for individual phospho- or glycolipids were found to be matrix-dependent to some extent. Presumably, the RTILs contribute to the analyte extraction from the tissue. Importantly, none of the tested RTILs showed analyte diffusion beyond the applied 15 µm step size of the MSI analysis. Comparison with H&E stained tissue showed that cerebellum structures were precisely imaged by the MS data. Due to the high viscosity of the gel-like matrix film layer, the ablation craters produced by the 30 laser shots applied per pixel (@30 Hz) were clearly visible hours after the measurements indicating the essential absence of substance migration over this time. At the same time, a very uniform signal response was obtained across the coated tissue as determined with liver homogenate. The described RTILS compromise a powerful alternative to conventional crystalline MALDI-MSI matrix preparations. In particular, they offer an easy sample preparation protocol and particular uniform sample coatings.

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Posternummer: 145

Visualizing metabolites in plant tissues using high-resolution atmospheric pressure scanning microprobe MALDI mass spectrometry imaging (SMALDI-MSI)

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Keywords: Plants, Mass spectrometry imaging, metabolites

Einleitung

The capabilities of multiplex, label-free imaging of chemical species in biosystems demonstrate the advantages of MS imaging in non-targeted metabolomics, lipidomics and proteomics. This tool has been utilized to investigate spatial distributions of molecules in mammalian tissue, whole body sections of animals, plants, insects and drug compounds in tissues. To resolve the complexity of biological samples, a high, subcellular spatial resolution, an improved mass accuracy and a high mass resolution are essential. Here we use an atmospheric-pressure scanning microprobe matrix-assisted laser desorption ionization mass spectrometry imaging (AP-SMALDI MSI) system with high resolution in mass and space, for its novel application in plants tissue obtained from crop plants- Brassica napus (rapeseed), Triticum aestivum (wheat seed) and medicinal plant Paeonia lactiflora (peony).

Experimenteller Teil

In order to obtain high quality images, sample preparation is one of the major challenges. We optimized several tissue sectioning methods (cryosectioning, embedding, tape-assisted methods etc.) compatible with MS imaging experiments. Matrix was applied using a high-resolution matrix-preparation unit (SMALDIPrep, TransMIT GmbH, Germany). Then, a high-resolution atmospheric pressure scanning microprobe matrix-assisted laser desorption/ionization imaging source ("AP-SMALDI10", TransMIT GmbH, Germany) with a Fourier transform orbital trapping mass spectrometer (Q Exactive, Thermo Scientific GmbH, Germany) was used to rasterize the tissue section [1]. High-quality m/z images (bin width of ± 5 ppm), showing highly resolved features (down to 5 µm pixel size) were generated using the software package 'Mirion'. Assignment of the compounds was based on high mass accuracy and MS/MS.

Ergebnisse und Diskussion

We used the AP-SMALDI10 system to visualize changes in metabolic processes during germination and fungal infection of crop plants, oilseed rape and wheat seed, respectively. From a single experiment, we were able to visualize more than 90 compounds. Metabolites of the sinapate ester metabolism having similar chemical structures were detected and visualized by MS imaging. Visualizing such metabolites simultaneously would be practically impossible with classical visualization techniques. In case of fungus-infected wheat seeds, we could visualize metabolites such as trimethylammonio butanoate, related to fungal infection. In peony, major tissue-specific metabolites, including monoterpene glucosides and gallotannins, were successfully visualized at the cellular level. AP-SMALDI MSI provides efficient technological advancements in the visualization of individual molecular species, beyond what is currently possible with other chemical imaging techniques.

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Native mass spectrometry analysis of norovirus glycan interaction

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Keywords: Native Mass Spectrometry, Noroviruses, Glycan, P domain, Binding.

Einleitung

Human noroviruses (HuNoV) are non-enveloped RNA viruses belonging to the Caliciviridae family and the main cause of viral gastroenteritis. The inability to culture HuNoV in cell lines hinders progress in viral entry studies. Murine noroviruses (MNV), closely related to HuNoV, are accessible for in vitro assays and small animal models to study norovirus infection. From the crystallographic structure, it is apparent that the norovirus capsid protein protruding (P) domain binds to glycans on the cell to mediate cell attachment. A detailed investigation of P domain-glycan interaction by native mass spectrometry (MS) is used to shed light on the mechanism behind viral cell entry.

Experimenteller Teil

Native MS is applied to investigate glycan binding to different MNV and HuNoV strains. Besides the known ligands, such as histo-blood group antigens and gangliosides, we also tested multivalent ligands based on the minimal binding motifs and proposed ligands, namely milk sugars. The number of binding sites per type of saccharide and potential conformational changes will be deduced and compared between the norovirus lineages.

Ergebnisse und Diskussion

The results will elucidate the saccharide binding profile of distinct noroviruses and deduce general binding principles in norovirus cell attachment. Preliminary data and the study design will be presented.

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Reproducing net charge states of proteins in solution phase with LILBID-MS

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Organisation(en): Goethe-Universität Frankfurt, Deutschland

Keywords: Native Mass Spectrometry, Net Charge, LILBID-QTOF

Einleitung

The net charge of proteins can play an important role in regulating their function. Nevertheless it is very difficult to determine the charge of protein complexes in solution. A method which mirrors the solution charge states of protein complexes would therefore be desirable.

LILBID (Laser-Induced-Liquid-Bead-Ion-Desorption)[1]is an ion source that transfers ions from solution into gas phase without addition of additional charges. We started to investigate the feasibility to investigate solution charge states of biomolecules with LILBID-MS by means of charge ladders. Comparing mass spectra of whole samples with spectra of ion chromatographically separated fractions shed new light on the observed charge states. Other groups reported earlier on the relationship between net charge and observed charge states using ESI-MS (Electrospray-Ionization)[2].

Experimenteller Teil

A piezo-driven droplet generator is used to produce droplets of 30µm diameter which are transferred to vacuum and irradiated by an IR laser operating at 2.94 µm, a vibrational absorption wavelength of water. This leads to an explosive expansion of the sample droplet and subsequently ions are released[1]. In order to analyze the charge ladders a LILBID source has been adapted to a modified Micromass QTOF-I (MS Vision) which offers a better resolving power compared to the 1st generation LILBID TOF.

Charge ladders have been produced by chemically modifying amino groups in the side chains of proteins. In our case the amino groups have been acetylated so the corresponding chains can't be protonated anymore.

Ergebnisse und Diskussion

LILBID-MS enables the investigation of a protein's net charge in solution. Changes in the pH value of the buffer immediately led to corresponding changes in the charge distribution observed in the mass spectra. A similar behavior can be seen using charge ladders. Varying degrees of acetylation revealed different charge states detected by MS as well. For example, in positive mode the net charge of hen egg lysozyme decreased with increasing number of caged amino groups. Exactly the same happened when changing the pH value from neutral to alkaline instead of using charge ladders.

The number of caged amino groups usually was smaller than the total amount of available ones. This points to accessible amino groups in case the protein is folded natively. Furthermore, the saturation curves in caging accessible amino groups differ for lysozyme and ubiquitin which could be explained by the respective protein structures. Attempts in separating individual degrees of acetylation by ion chromatography revealed a clear relationship between the number of caged groups and therewith the protein net charge and the detectable charge states in MS.

Overall we aspire to determine the correlation between solution net charge and charge states observed with LILBID MS under different solution conditions.

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Posternummer: 148

Dynamics of non-structural polyprotein 7-10 procession and formation of a replication-transcription complex of SARS Coronavirus

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Keywords: Replication, Virology, Structural Biology, Native MS

Einleitung
Emerging severe acute respiratory syndrome (SARS) coronavirus in southern China and the following expansion to 29 countries within half a year was responsible for more than 8000 infections with a case fatality rate of 9.6 % in early 2003. Focusing world media spotlights and insecurities lead to financial damage of several billion USD even well beyond the affected areas.

Coronaviruses ORFs 1a and 1ab encode for polyproteins (pp) that are further processed by viral proteases into 16 non-structural proteins (nsp). Replication and transcription of the viral ss(+)-RNA genome is controlled by the viral nsps, yet the interaction of the nsps themselves and with viral RNA or host cell factors is not well understood.

Experimenteller Teil
In order to solve the assembly of a large putative replication-transcription complex (RTC) the polyprotein 7.10 digestion by the viral protease nsp5 into nsp7, nsp8, snp9 and nsp10 was observed via ESI-Q-ToF mass spectrometry. Prior to measurement the proteins of interest were buffer exchanged into ammonium acetate which preserves non-covalent interactions and thus physiological fold and oligomerization qualifying for the term native mass spectrometry. Preparation of the experiments comprised of recombinant E. coli expression, affinity and size exclusion chromatography purification pp7.8, pp7.9 and pp7.10 and nsp5.

The polyprotein/ protease mix was prepared at different ratios and buffer conditions for end point as well as time-resolved analysis of the intermediates and matured procession products.

Ergebnisse und Diskussion
The order of digestion of the single cleavage sequences could be observed. The protein is digested from the N-terminus starting cutting off the his(6x)-tag followed by release of nsp10 then nsp9 and finally leading to cleavage between nsp8 and nsp7. While nsp7 and nsp9 were only detected as monomers, a several nsp7/nsp8 oligomers could be detected that will be of interest for the assembly of the RTC. The dimer of nsp8 was found frequently. Furthermore a tetramer of nsp7+8 (2:2) was found and confirmed via collision induced dissociation (CID) tandem mass spectrometry. Previously, nsp7/8 structures have been reported as (2:1) trimer (Xiao, 2012) and (8:8) hexadecamer (Zhai, 2005) acquired by X-ray crystallography. This complex is of particular interest because it inhibits unique multimeric RNA polymerase capable of both de novo initiation and primer extension. Future experiments will aim towards understanding the influence of nucleic acid binding on complex formation. Additionally, we want to show first data on ion mobility mass spectrometry (IMMS) that allows us to calculate the collisional cross section (CCS2) of the polyproteins and the nsps as well as the newly formed oligomers.

Referenzen

Assembly pathway and shape of a lipid-associated protein complex

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Keywords: Native Mass Spectrometry, Endocytosis, Surface-induced dissociation, Lipid-binding

Einleitung

Clathrin-mediated endocytosis is the major trafficking route from the plasma membrane to the cytoplasm and thus indispensable for a vast number of cellular processes. Remodeling of membrane curvature during endocytosis requires a mechanical force to be transmitted to the membrane. In this context, clathrin adaptor proteins act as linkers between clathrin coat, plasma membrane and actin cytoskeleton. Attachment to the plasma membrane occurs via the membrane associated ANTH and ENTH domains of two different clathrin adaptor proteins. These were shown to co-assemble in the presence of the phospholipid PI(4,5)P2 [1].

Experimenteller Teil

Native mass spectrometry was used to investigate complex stoichiometries for co-assembled ANTH/ENTH/PIP2-complexes not only for proteins from S. cerevisiae and C. thermophilum but also for human proteins. Structural information was gathered using ion mobility mass spectrometry (IMMS) and the gas-phase dissociation techniques CID (Collision induced dissociation) and SID (surface induced dissociation).

Ergebnisse und Diskussion

ANTH and ENTH proteins from S. cerevisiae and C. thermophilum co-assemble in the presence of the phospholipid PI(4,5)P2 to complexes with the stoichiometries 6+6 and 8+8. Two different ENTH-domains were shown to form equivalent complexes in yeast. Formation of chimeric assemblies highlights the universal principle of clathrin adaptor protein assembly. Time-course experiments reveal the conversion of the intermediate 6+6 complex to the more stable 8+8 form, resulting in a model of the assembly pathway. Human ENTH proteins were found to form a hexamer that serves as assembly platform for larger ANTH/ENTH/PI(4,5)P2 complexes.

From CID and SID IMMS measurements it can be concluded that ANTH proteins are located in the periphery of ANTH/ENTH/PI(4,5)P2 complexes and statements about the protein-protein interfaces and lipid binding sites can be made. Furthermore, the importance of phospholipids for the overall shape of the complex is shown.

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Application of Isotope Dilution Mass Spectroscopy for the Method Development towards Isotope Labeled Algae Biotoxin Certified Reference Materials

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Organisation(en): Sigma-Aldrich, Merck, Switzerland

Keywords: Algae toxins, CRMs, IDMS

Einleitung

The presence of algae biotoxins in shell fish and other sea food is an emerging problem in food testing. Fast and sensitive LC-MS methods were established very recently [1]. Therefore, the access to well characterized reference materials for a precise quantitation of these toxins is now a crucial need of the testing laboratories.

Experimenteller Teil

The system caffeine / 13C3-caffeine was used to test the performance of the LC-MS system for IDMS measurements. The mass fractions of caffeine and 13C3- caffeine were determined by 1H-HP®-qNMR beforehand. Two separate aqueous caffeine solutions with final concentrations of 20 µg/g were prepared at equal and different ratios. The LC-MS system was set up in ESI(+) scanning mode with a mass range +/- 5 Da higher and lower than the [M+H]+ ions of the labeled and non-labeled compound. The results were achieved by preparing and measuring six individual samples in sixfold and applying the separation conditions as described in [1]. Comparable parameters were chosen for the analysis of Neosaxitoxin and the stable isotope labeled 15N7-Neosaxitoxin analog.

Ergebnisse und Diskussion

The mass fraction of 13C3-caffeine as determined by LC-MS was 98.4% while the total caffeine content in the solid material determined by 1H-HP®-qNMR was 98.9 %. Gravimetric dissolving of the neat products led to a final concentration of 19.80 µg/g 13C3-caffeine referring to the weight of solid material and solvent. Basic reverse IDMS with caffeine as reference gave a concentration of 19.75 µg/g for the 13C3-caffeine solution. An intra-laboratory comparison (3 persons, 3 days, 2 balances, 2 different reference solutions) of independently prepared caffeine/13C3-caffeine standards and sample solutions showed an overall variation +0.2/-0.2 µg/g of the measured concentrations. After this demonstration of the robustness of the method, labeled 15N7-Neosaxitoxin was diluted and analyzed the same way. A corresponding reference solution of the non-labeled Neosaxitoxin was analyzed quantitatively by 1H-HP®-qNMR. With that reference standard the received certified concentration of 10.00 ± 0.41 µg/g for the labeled saxitoxin derivative was cross-checked by LC-MS and yielded in a concentration of 9.91 ± 0.2 µg/g. Both mean values are in agreement with respect to their assigned uncertainties.

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Die Analyse von Caseinen mittels zweidimensionaler HPTLC-MALDI-(Imaging)-MS

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Keywords: MALDI-Imaging, HPTLC, TLC-MS, Peptide

Einleitung

Proteine und Peptide haben weitreichende biologische Funktionen und daher für die Lebensmittelindustrie eine hohe Bedeutung. Der massenspektrometrischen Analyse gehen meistens elektrophoretische und/oder chromatographische Trennmethoden voraus. Die Verwendung unterschiedlicher Verfahren für dieselbe Probe kann dabei zu Abweichungen der Ergebnisse führen[1]. Darüber hinaus können post-translationale Modifikationen große Herausforderungen an die Trennung der Peptide stellen. Artifizielle Modifikationen resultierend aus der der Be-/Verarbeitung von Lebensmitteln sind dabei kaum berücksichtigt.

Die Hochleistungsdünnschichtchromatographie (HPTLC) in Kopplung mit der Massenspektrometrie stellt eine vielversprechende Ergänzung zu den etablierten Analysenmethoden dar. Eine hohe Zahl der Freiheitsgerade durch große Variabilität in der mobilen und der stationären Phase sowie zweidimensionale Entwicklung ermöglichen die Trennung auch komplexer oder matrixbelasteter Proben. Die Detektion und Charakterisierung erfolgt über Fluoreszenz, Derivatisierungsreagenzien und/oder verschiedene massenspektrometrische Technologien[2,3].

Experimenteller Teil

Die in dieser Studie modellhaft verwendeten Caseine wurden durch alkalische Fällung aus kommerziell erhältlicher Kuhmilch gewonnen, durch Trypsin in Peptide gespalten und anschließend durch SPE extrahiert. Die Trennung erfolgte auf HPTLC Kieselgel 60 F254 MS-grade für MALDI 5x7,5 cm Platten (Merck) in einer und zwei Dimensionen mit basischer, bzw. saurer mobiler Phase. Für den optischen Vergleich zwischen traditioneller Derivatisierung durch Fluorescaminund der Massenspektrometrie wurden zwei identisch entwickelte Platten verwendet. Eine HPTLC-Platte wurde nach der Derivatisierung bei λ=366 nm fotografiert und die zweite, identische HPTLC-Platte massenspektrometrisch durch MALDI-Imaging-MS mit einem ultrafleXtreme MALDI-TOF/TOF-MS (Bruker Daltonik) analysiert. Zuvor erfolgte ein zweimaliges Tauchen der speziell für die Massenspektrometrie entwickelten HPTLC-Platte in 2,5-Dihydroxybenzoesäure (MALDI-Matrix). Im Anschluss an das Imaging wurden MS/MS-Messungen der detektierten Peptide durchgeführt.

Ergebnisse und Diskussion

Die komplexe Mischung der Peptide konnte mit hoher Bandenschärfe in eindimensionaler HPTLC zu großen Teilen getrennt werden. Durch die Vielzahl der Peptide und der ähnlichen chemischen Eigenschaften einzelner Peptide ist eine vollständige Trennung aller Peptide voneinander in einer Dimension allerdings nicht möglich. Dies wird durch den optischen Vergleich der zwei identisch entwickelten Platten deutlich. Im MALDI-Image sind deutlich mehrere Massen an der Position einzelner Fluoreszenzbanden zu erkennen. Durch die Erweiterung der Chromatographie in eine zweite Dimension kann ein Teil der in erster Dimension nicht getrennten Peptide zusätzlich separiert werden.

Auf einer einzelnen TLC-Platte können durch die MALDI-Imaging-MS über 85 Peptidspots detektiert werden. Der Vergleich dieser Peptidmassen mit denen aus einem in silico-Verdau der vier bekanntesten Caseine αS1-, αS2-, β- und κ-Casein erlaubt die Identifizierung der Proteine mit einer Sequenzabdeckung von 40 – 95%.

MS/MS-Messungen der detektierten Peptide waren möglich. Allerdings konnte nur eine geringe Anzahl an Peptiden durch de novo Sequenzierung oder Datenbanksuche eindeutig identifiziert werden, da die Qualität der Fragmentspektren in der Regel zu gering war. Hervorzuheben ist dabei ein Phosphopeptid bei dem die Position der modifizierten Aminosäure eindeutig bestimmt werden konnte. Die detektierten Phosphopeptide weisen niedrige Rf-Werte auf.

Die Kopplung mit MALDI-(Imaging)-MS erhöht die Menge an Information von einer einzelnen HPTLC-Platte gegenüber Fluoreszenzdetektion deutlich. Einzelne Peptide konnten durch MS/MS-Messungen vollständig charakterisiert werden und zeigen mit der Gesamtzahl detektierter Peptidmassen das Potential der Methode. Optimierungen, wie die Erhöhung der Homogenität der Matrix-Schicht und spezifische Modifikationen der HPTLC-Platte, können zu einer weiteren Verbesserung der Sensitivität, der Auflösung und des Signal-zu-Rausch-Verhältnisses beitragen.

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Posternummer: 152

Non-invasive monitoring of microbial cultures by HS-SPME-GC/MS: a pilot study.

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Keywords: Headspace SPME, volatile organic compounds, microorganisms, food industries, biotechnology

Einleitung

The cultivation of microbial cultures under controlled conditions in a bioreactor plays a central role for many production processes in biotechnology or in the food industries. A variety of different analytical parameters (cell density, pH value etc.) has to be monitored for an efficient process management. There are invasive as well as non-invasive sampling methods available, which are tailored for the individual analytical tasks, e.g. taking a liquid sample from the bioreactor for the identification of microorganisms by standard methods. Non-invasive methods like IR spectroscopy for determining carbon dioxide levels on-line in exhaust gas released from the bioreactor are applied whenever possible, because they do not directly interfere with the cultivation process and have a lower contamination risk.

Experimenteller Teil

In principle, the release of characteristic volatile organic compounds (VOCs) from cultivated microorganisms could contribute to species identification as well as process monitoring tasks. In the pilot study presented here, we propose the use of adapted HS-SPME-GC/MS methods for the analysis of characteristic VOCs emitted into the headspace of prototypic bacterial cultures. First experiments aiming at the identification of volatile compounds suitable as biomarkers were performed on an Agilent 6980 GC system coupled to a 5973N mass selection detector.

Ergebnisse und Diskussion

Results of the initial screenings for VOCs released by bacteria cultivated in liquid medium point to a couple of suitable VOC species, which could be indeed used as markers for the characterisation of bacterial cultures under the conditions used here. In the next steps optimisation of the cultivation conditions as well as the analytical method will be combined with in-depth database research in order to set-up the full chain of sampling, measurement and computer-assisted data evaluation. Future applications of a non-invasive bioreactor monitoring by HS-SPME-GC/MS could be extended to the analysis of pathogenic microbial cultures in critical industrial settings.

Referenzen

Real-time food authentification with a miniature mass spectrometer using statistical data analysis

Autoren: Gerbig, Stefanie; Neese, Stephan; Lotze, Christian; Spengler, Bernhard; Schulz, Sabine

Organisation(en): Justus Liebig University Gießen, Deutschland

Keywords: Miniature mass spectrometry, food analysis, adulteration, principal component analysis

Einleitung

Since the number of food items in global trade is steadily increasing, the control of regulations regarding crop treatment and food production has become more and more important. Food fraud and adulteration are found on a regular basis. Mass spectrometric techniques were established, that allow to control food authenticity, employing methods for marker detection as well as pattern analysis. Since these analyses should ideally be carried out directly on site, a portable mass spectrometer was utilized to obtain spectra from different food items. Data was processed using principal component analysis (PCA) and different classifiers.

Experimenteller Teil

The portable mass spectrometer "Mini 11", developed at Purdue University consists of a linear ion trap which provides unit mass resolution and a mass accuracy of 1-2 u.[1] The size of the Mini 11 is approx. 20x30x15 cm3, its weight is 5 kg. Data was recorded in positive ion mode in the m/z range of 70 to 800 using electrospray and low temperature plasma ionization. The recorded raw data were directly processed with the home-built software package "MSClassifier". This program performs PCA on spectra of known food items which can be stored in and selected from a repository and allows for spectra classification of unknown food items in real-time using Mahalanobis distance and the linear discriminant analysis coefficient as classifiers.

Ergebnisse und Diskussion

Statistical analysis of Mini 11 data was carried out for several food items using various ionization techniques. At first, measurement of different juice and wine samples proved the applicability of the method and instrumentation for food authentification. These experiments were conducted using electrospray ionization of diluted samples. Sets of five mass spectra were averaged for analysis. Spectra of the different food items showed good separation after statistical analysis and formed separate data groups. Subsequent measurements comprised a set of three milk samples (goat milk, cow milk, soy milk). Approx. 200 mass spectra were recorded of each sample with ESI in full scan mode to enable reasonable statistical data analysis. Again, spectra classes were clearly distinguished and all three kinds of milk showed separated data point groups. Data was subsequently used to classify new spectra recorded from goat milk in a real-time experiment. 99 % of the recorded single spectra were classified correctly. Furthermore, a low temperature plasma (LTP) was used as ionization source to analyze different kinds of coffee beans. For this experiment, single spectra were recorded from Arabica and Robusta coffee and subjected to statistical analysis. Results revealed a high similarity of spectra, but nonetheless, a cross-validation experiment showed a correct classification for 90% of Robusta bean spectra and for 96% for Arabica bean spectra, based on the coefficient of linear discriminant analysis. These measurements indicated that even low mass resolution and low mass accuracy data, as recorded from miniature instruments, can successfully be used for pattern analysis and therefore aid in on-site identification of food items and the discovery of food fraud.

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Targeted and non-targeted screening of biocides in urban stormwater runoff

Autoren: Quilitzki, Julia (1); van Baar, Patriicia (1); Dünnbier, Uwe (1); Wicke, Daniel (2); Bromirski, Maciej (3); Scheibner, Olaf (3)

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Keywords: high resolution mass spectrometry, Orbitrap, unknown screening, degradation products

Einleitung
Untreated stormwater runoff can be an important source of organic micropollutants entering urban surface water through separated sewer systems. To investigate the extent of this type of pollution, a one year monitoring program was conducted in the city of Berlin to analyze micropollutants in urban stormwater runoff. A set of biocides and

plant production products were chosen for quantification

Experimenteller Teil
Five sampling points were installed in separate storm sewers of five different catchment types representing 85% of the connected impervious area of Berlin: single houses with gardens, roads, old building-, newer building- and commercial areas.

Target and non-target screening was performed on an E Quan Max Plus (Thermo Fisher Scientific) online SPE system which is linked to a Q Exactive Focus mass spectrometer (Thermo Fisher Scientific). The data processing and evaluation was performed with TraceFinder 3.3 and Sieve 2.0 (Thermo Fisher Scientific). A home built database with 2188 entries was applied using isotope pattern- and fragment matching for the compound identification.

Ergebnisse und Diskussion
This study shows that suspect screening analysis is an efficient method for detection of unknown compounds (for example simazine, nicotine, benzalkoniumchloride).

The high measured concentrations of substances shows that stormwater runoff can be an important entry pathway for micropollutants to water bodies. Results also indicate that it is necessary to consider the different urban catchment types with its specific compounds.

Referenzen

Posternummer: 155

Comparison between On-line and Off-line measurements of microparticles from air, rock and snow water

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Keywords: mass spectrometry, atmospheric aerosols, chemical composition, single particle analysis

Einleitung

By using the compact laser mass spectrometer LAMPAS 2 during the measuring campaign CLACE-1 it was shown that the chemical composition of particles in the free troposphere at the Jungfraujoch in the Swiss alps strongly depends on their transportation pathways (Hinz et al., 2005). The influence of wind and weather leads to particle classes which are characteristic for different natural and anthropogenic particle sources. On-line measured data are more likely reflecting the natural state of such particles in the atmosphere, compared to off-line measurements. The influence of the surrounding environment at the measurement site on the particle populations, even at such isolated places as the Jungfraujoch, has not really been considered yet and was therefore investigated in this study.

Experimenteller Teil

Samples of snow water from a measuring campaign in 2000 were directly analyzed by an ICP mass spectrometer to determine the elemental composition of soluble components. Drying residues of these samples and grinded rock samples from the top region of the Jungfraujoch were measured with the compact mobile laser mass spectrometer LAMPAS 3 (19” rack, 150 cm in height). On-line analysis of atmospheric aerosol particles from the free troposphere was performed with an earlier version of the instrument (LAMPAS 2) during the same measuring campaign in 2000. Mass spectrometric data was then evaluated using a fuzzy clustering algorithm to characterize particles. Results were compared to determine the influence of different kinds of particle sources at the Jungfraujoch.

Ergebnisse und Diskussion

On-line measurement data of particle populations at the Jungfraujoch from the year 2000 were compared to the composition of rock and snow water samples from the same location. Off-line analysis data were acquired with the LAMPAS 3 instrument to be comparable with the on-line data sets produced with the LAMPAS 2 instrument in 2000. The total elemental composition of the soluble components in the snow water samples shows a high calcium content. Including the observation of a calcium rich class of particles, representing 16 % of the total particle composition of the snow water residues, these particles were identified as sediment particles originating from limestone. Additionally some mineral particle components of the rock samples from the top region of the Jungfraujoch were recovered in about 1 % of the mass spectrometric data of the snow water residues. Transportation especially of limestone-like particles from the lower regions of the Jungfraujoch is possible due to erosion by wind and water. The results allow for a better assessment of the environmental influences that affect the particle populations at the Jungfraujoch.

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Does the bioavailability of DDT residues in soils depend on agricultural practice?

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Organisation(en): Hochschule Mannheim, Deutschland

Keywords: DDT, mobility, pesticides, soil, GC-MS

Einleitung

Almost 40 years after the ban of DDT for agricultural use in Western Europe, its long half-life still is an environmental issue [1]. As a result, soil contamination levels of up to 200 ppb of DDT, DDE and DDD (DDX) are still present [2]. Cultivation on these soils may lead to bioaccumulation [3], and therefore transition into the food chain [4]. Recent studies on phytosanitation strategies give rise to the assumption that the bioavailability of DDX increases upon treatment of soils with surfactants (Lit?). However, so far no studies have evaluated potential effects of the repeated application of typical surfactant-rich pesticide formulations.

Experimenteller Teil

In cooperation with an industry partner, a 1ha field trial was started in May 2013 with 10.000 plants of the cultivars Curcubita cv. Howden and Cucurbita cv. maxima in 2014. For this trial, a typical organic treatment protocol was compared to a conventional protocol. A section without plant protection was used as a control. During the vegetation period from May to September, samples were collected in regular intervals from the soil and plant parts, in particular before and after pesticide application. Extraction of the samples was performed by accelerated solvent extraction (ASE)[5], followed by a modular sample clean-up step. DDX determination was performed by GC-MS analysis (Agilent 6980/5973), operated in EI mode.

Ergebnisse und Diskussion

Residual soil levels in the trial field were comparable in 2013 and 2014 and ranged between 20-60 µg/kg with a normal distribution over all trial sections. In contrast, the DDX levels in the plants cultivated in 2013 differed significantly from the 2014 harvest. In the cultivar Curcubita cv. Howden accumulations of up to 200 ppb DDX were detected. The highest concentrations were found in the stem of the plant, while fruits and seeds contained significantly lower amounts. In the 2015 cultivar Cucurbita maxima, DDX concentration were clearly lower, on average 40 ppb. It is yet unclear whether the difference in uptake rate is depending on the different plant cultivar or if a fluctuation in temperature leads to uptake difference. The climatic conditions differed significantly in 2013 and 2014, in particular in sunshine duration and precipitation amount. Interestingly, soil samples tentatively showed differences in the extractable content of DDX before and after pesticide treatment. One week after the treatments, the DDX content was higher than before, when analyzing samples from the same sampling sports. This gives rise to the assumption that pesticide application may have an effect on the mobility of DDX and consequently also to the bioavailability in plants.

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Method development for the identification of novel brominated flame retardants using a Q Exactive HRAM mass spectrometer

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Keywords: Method development, NBFRs, Orbitrap, APCI, Compound Discoverer

Einleitung

Due to legislative restrictions on manufacture and use of some brominated flame retardants (BFRs), several new chemicals (NBFRs) have been developed. To explore their presence in different environmental compartments and ultimately understand their environmental fate, analytical methods for targeted analysis are required [1]. Classically these compounds are determined by GC-based instrumental methods. In recent years, LC-based methods coupled to low resolution mass spectrometers have also been developed [2]. Advances in high resolution mass spectrometry facilitate accurate measurements and identification of unknowns, including degradation and transformation products. Moreover, bromine isotopic pattern analysis as well as the use of mass defect plots and filters, helps identify relevant substances, with such techniques starting to be more commonly used in environmental science [3].

Experimenteller Teil

Dust samples were collected according to an established protocol [4]. Extraction was conducted using a Thermo Scientific™ Dionex™ ASE™ 350 accelerated solvent extractor and in-cell cleanup. Final extracts were separated on a Thermo Scientific Accucore™ RP-MS 100x2.1mm, 2.6µm column on a Thermo Scientific Accela™ HPLC system (Accela™ 1250 Pump and Open autosampler), using a 15 min. gradient elution program with water (mobile phase A) and methanol (mobile phase B) at a flow rate of 400 µl/min. Samples were analyzed on a Q Exactive™ mass spectrometer with an APCI source. Raw data files were processed using Thermo Scientific Compound Discoverer™ version 2.0 software. In addition, mass defect plots were created using Microsoft® Excel to visualize the presence of brominated compounds.

Ergebnisse und Diskussion

The use of different solvent mixtures in the accelerated solvent extractor was investigated to obtain the optimal extraction results for the analytes of interest. Extraction solvents include hexane, dichloromethane and acetone. Further in-cell clean up using silica and florisil was performed to reduce matrix interferences.

A HPLC gradient elution program and APCI values were optimized based on the measurement of reference standard solutions. Extracted samples were then measured with the optimized conditions.

Initially, Full Scan experiments were conducted to obtain a general overview of the presence of compounds of interest in the samples. The use of high-resolution accurate mass (HRAM) instrumentation facilitates identification of targeted compounds and unknowns by means of selectivity, elemental compositions and isotopic pattern scoring. Later, confirmation of compounds was conducted using MS2 fragmentation spectra and measured reference standards. Several BFRs and NBFRs were identified in the dust samples.

Thermo Scientific Compound Discoverer™ software was successfully employed as a tool for identification and screening of possible further compounds of interest. The workflow included tools, such as mass defect filtering, isotopic pattern scoring and an unknown detector.

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Pesticides Target Screening with an atmospheric pressure chemical ionisation GC coupled to high-resolution Q-TOF-MS

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Keywords: Target Screening

Einleitung
Accurate mass QTOF-LC/MS with electrospray ionisation for target pesticide screening enables the identification of hundreds of pesticides in a single run. On the other hand, GC/MS is well suited to these compounds and generally exhibits less matrix effects whilst producing lower chemical background. In this study, we combine the advantages of GC and QTOF technologies using a novel GC-APCI interface to a QTOF mass spectrometer. Specifically, we report the application of a GC-APCI source coupled to a high resolution QTOF-MS to extend the scope of trace pesticides screening in standard mixtures as well as spiked samples, food and water samples.

Experimenteller Teil
Analyses were performed using a 60 Pesticide StandardMix [1]. Also standards were spiked into fruit and vegetable matrices. 1 µl ofeach sample was injected and separated using a Restek Rxi-5ms capillary (30m,0.25 mm ID, 0.25 µm film). The GC column was interfaced to an Q-TOF-MS (ImpactII ,Bruker Daltonics) with a GC-APCI source operated in both positive andnegative ionisation mode. Data were acquired from 50-1000 m/z at minimum of 4Hz. All files were acquired with automatic mass calibration at the beginning ofeach GC/MS run with a perfluorinated calibration standard.

Ergebnisse und Diskussion
The pesticide standard mix consists of a set of 60 representative pesticidesselected according to their relevance in today´s routine food analysis andaccording to their chemical characteristics as molecular mass, chemicalcomposition, their polarity and volatility. The mix contains amongst others:Azinphos-Methyl, Chlorpropham, Diazinon, Dimethoate, EPN, Imazalil,Myclobutanil, Pirimicarb [1]. Here we developed a base method using GC-APCI-Q-TOF which in future should bedeveloped further towards a more comprehensive GC-APCI-QTOF method coveringhundreds of pesticides. All data were generated using automated mass calibration during each GC/MS run.This enables a mass accuracy at 3 ppm throughout all samples and would allowthe assignment of unknown components.The GC-APCI-Q-TOF-MS system was calibrated for quantification with the 60pesticide standard in the concentration range of 0.05 to 500 pg/µl. Limits ofquantification (LOQ) for most of the pesticides were found to be in the rangebelow 10 pg/µl with RSDs between 5 and 10 % (N=3). Additional analytical datawill be presented to further characterize the evaluation of this analyticalapproach. Additional results will be shown from food, water samples, and pesticides thatwere spiked into fruit standards, extracted and analysed.

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Potential of collision cell ICP-MS and ICP-MS/MS for sensitive and interference free element and elemental speciation analysis of environmental samples

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Keywords: ICP-MS/MS, Speciation, Environment

Einleitung

Anthropogenic activities result in the continuous release of contaminants such as trace elements and their species into the environment. ICP-MS and its hyphenated techniques have become the methods of choice for the accurate determination of such environmental contaminants [1]. Elemental analysis in environmental samples is strongly hampered by the formation of interfering polyatomic ions due to reactions between the sample matrix and the plasma gas. In particular the introduction of collision and reaction cell as well as ICP-MS/MS helped to overcome the problems related with the analysis of such samples. This contribution will highlight the possibilities of ICP-MS-MS combined with different sample introduction approaches for the analysis of challenging environmental matrices such as sediments, biota or water samples.

Experimenteller Teil

An Agilent 7700 cs collision cell ICP-MS as well as an Agilent 8800cx ICP-MS/MS has been used during all experiments. To overcome the interference problem related with the analysis of most elements when analyzing environmental matrix cell gases such as He, H2, NH3 and O2 have been evaluated in combination with different sample introduction approaches.

To demonstrate the strength of recent ICP-MS based techniques different application examples ranging from element analysis in different sample matrix to ultra trace speciation analysis using GC and LC-ICP-MS will be demonstrated.

Ergebnisse und Diskussion

It will be demonstrated that the developed approaches can be applied for the accurate quantification of trace metals and their species in various kind of sample. The application of different cell gases allowed the interference free analysis of strongly interfered elements such as S, P, As, Se and many others with detection limits down to the low ng/L range. For the Analysis of the mentioned elements best results have been achieved using O2 as cell gas as well as the MS/MS configuration. The mass shift reaction with O2 allows to shift the mass of the interfered ions into a m/z region with is less interfered via the oxidation of the targeted ions (e.g. 32S+ and 16O will form 32S16O+ which can be detected at m/z 48, which is not interfered . In particular the application of ICP-MS/MS helped to improve the recovery for many elements due to the improved interference handling, which is a consequence of the newly available cell gases as well as the MS/MS configuration of the mass analyser. Overall the MS/MS approaches allows to set up suitable detection schemes for nearly every element of the periodic table allowing their sensitive and interference free determination.

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Selektive und sensitive Detektion und Quantifizierung von Persistent Organic Pollutants (POPs), inklusive der Dioxine, mittels Atmosphärendruck Gaschromatographie - Tandem Massenspektrometrie (APGC-MS/MS)

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Keywords: Persistent Organic Pollutants (POP), Polychlorierte Dibenzo-p-Dioxine (PCDD), Atmosphärendruck Gaschromatographie, APGC-MS/MS

Einleitung

Polychlorierte Dibenzo-p-Dioxine (PCDDs) und Furane (PCDFs) sind unter dem Annex C der Stockholm Convention von 2004 gelistet. In den letzten Jahren hat das Umweltprogramm der Vereinten Nationen (UNEP) seinen Fokus auch auf die Entwicklung von analytischen Methoden zur Detektion von POPs gelegt.[1] Dioxine werden gemeinhin als die größte analytische Herausforderung der POPs angesehen.

Atmosphärendruck GC (APGC) wurde in den 1970iger Jahren entwickelt.[2] In letzter Zeit wird es als Alternative zu hochauflösender EI-GC-MS und EI-GC-MS/MS wiederentdeckt. Vorteil dieser Technik ist die im Vergleich sanfte Ionisierung. Viele POPs bilden nur das Molekül-Ion bzw. Quasi-Molekül-Ion unter APGC-Bedingungen. Dadurch erhöht sich die Sensitivität im MRM/SIR Modus, weswegen APGC ein großes Potential für die selektive und sensitive Analyse von Dioxinen hat.

Experimenteller Teil

In dieser Arbeit wurde APGC gekoppelt an ein Xevo TQ-S Tandem-Quadrupol Massenspektrometer im positiven MRM Modus unter trockenen N2-Bedingungen für die Analyse von POPs eingesetzt. Auf einem Agilent 7890A GC wurde eine DB-5MS UI Kapillarsäule (Dimensionen 60 m x 0,25 mm x 0,25 µm, J&W Scientific, Folson, CA/USA) für die Trennung verwendet. PCDDs und PCDFs Standards wurden von Wellington Labs bezogen.

Die APGC-Ionisierung ist mit APCI vergleichbar. Das bedeutet, dass eine Corona Nadel ein Stickstoff-Plasma generiert in dem über zwei verschiedene Ionisierungsmechanismen - Charge Transfer und Protonierung - Molekül-Ionen entstehen. Es ist möglich mit unterschiedlichen Quellen-Bedingungen zwischen beiden Mechnismen zu wählen oder einen Mix aus beiden zu generieren, je nach Chemie des Zielanalyten.

Ergebnisse und Diskussion

Unter "trockenen" Bedingungen in der Quelle werden die PCDDs und PCDFs durch den Charge-Transfer Mechanismus ionisiert. Die Ionisierung in der APGC wird generell als gering energetisch im Vergleich zur Elektronenstoß-Ionisierung angesehen. Die sich daraus ergebende hohe Abundanz der Molekül-Ionen führt zu einer eindrucksvollen Verbesserung der Sensitivität eben auch für die untersuchten PCDDs und PCDFs.

Kalibriergeraden wurden in einem Konzentrationsbereich von 10 fg - 40 pg absolut auf der Säule aufgenommen. Die Korrelationskoeffizienten und %RSD der Kalibierung waren > 0,998 bzw. < 10% für alle 17 Verbindungen.

Die Untersuchung der Reproduzierbarkeit der berechneten Konzentrationen und der Ionenverhältnisse von Quantifizierungs- und Qualifizierungsübergang ergaben ebenfalls sehr gute Ergebnisse.

Die Daten zeigen also, dass die Sensitivität von APGC-MS/MS für chlorierte Dioxine und Furane vergleichbar mit hochauflösender GC-MS ist, die zur Zeit als Standardanalysemethode eingesetzt wird. APGC kann damit als alternative Methode für die Analyse der in der Stockholm Convention gelisteten Dioxine gesehen werden. Die Möglichkeit das Xevo TQ-S Tandem-Massenspektrometer mit nur geringem Umbauaufwand sowohl für LC-MS/MS als auch für APGC-MS/MS einzusetzen erweitert die methodische Vielfalt im Analytiklabor speziell bei der Bestimmung von POPs.

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Characterization of the mass dependent transmission efficiency of a CIMS

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Keywords: Mass dependent transmission efficiency, mass discrimination, Chemical Ionization mass spectrometry

Einleitung

Knowledge about mass discrimination effects in a Chemical Ionization Mass Spectrometer (CIMS) is crucial for quantifying e.g. the recently discovered Extremely Low Volatile Organic Compounds (ELVOCs) [2] and other compounds for which no calibration standard exists so far. Here, we present a simple way of estimating mass discrimination effects of a nitrate based Chemical Ionization Atmospheric Pressure interface Time of Flight (CI-APi-TOF) mass spectrometer.

Experimenteller Teil

Characterization of the mass discrimination is achieved by adding different perfluorinated acids to the mass spectrometer in amounts sufficient to deplete the primary ions significantly. The relative transmission efficiency can then be determined by comparing the decrease of signals from the primary ions and the increase of signals from the perfluorinated acids. This method is in use already for Proton Trasnfer Reaction-MS, however its application to a CI-APi-TOF brings additional difficulties, namely clustering and fragmentation of the measured compounds, which can be treated with statistical analysis of the measured data. We also compare this method to a transmission estimation obtained with a setup using an Electrospray Ion source, a High Resolution Differential Mobility Analyzer and an electrometer.

Ergebnisse und Diskussion

Both methods give different transmission curves, indicating non-negligible mass discrimination effects of the CI source. The absolute transmission of the instrument without the CI source was estimated with the HR-DMA method to plateau between the m/z range of 127 and 568 Th at around 1.5 %, however for the CI source included, the depletion method showed a steady increase in relative transmission efficiency from the m/z range of the primary ion (mainly at 62 Th) to around 550 Th by a factor of around 5.

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Characterization of Electrochemically Decomposed Lignin Using Liquid Chromatography-High Resolution Mass Spectrometry

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Organisation(en): Universität des Saarlandes, Deutschland

Keywords: high-resolution mass spectrometry, lignin analysis, data simplification

Einleitung

Research on lignin decomposition is of great importance because of the potential to recover valuable chemicals from the lignin polymer waste. Several decomposition techniques have been applied for selective degradation of lignin. Electrochemical decomposition, in particular by means of protic ionic liquids (PIL), has extended the range of accessible molecules considerably, because novel degradation pathways can be accessed. To fully characterize these pathways from the highly complex mixtures of structurally diverse decomposition products, selective liquid chromatography using novel stationary phases and detailed high resolution mass spectrometry were applied in this study.

Experimenteller Teil

Lignin (5% w/w) was dissolved in triethylammonium methane sulfonate and electrochemically decomposed. After several extraction and evaporation steps, the resulting solid was dissolved in ACN/H2O (10:90 v/v) and chromatographically separated. The columns used were commercially available (C18 and propylphenyl/C18) as well as custom-made, self-packed ionic liquid based-stationary phases. A post-column flow of toluene (10%) was added and transferred to a Bruker solariX 7.0T FTICR mass spectrometer using atmospheric pressure photoionization (APPI). The complex data sets were interrogated by using enhanced mass defect filter strategy.[1]

Ergebnisse und Diskussion

In our experiments, electrochemical decomposition broke down the lignin polymer to products of molecular weights between 200 and 900 Da. The majority of the signals were in the m/z range between 200 to 450, representing dimeric, trimeric and low mass tetrameric compounds. However, several other reaction types (oxidation, reduction, repolymerization and combinations of the three) extended the product range to compound classes unique for electrochemical decomposition. Biphenylene-type degradation products as well as unsaturated derivatives of more common breakdown products were detected. In addition, chromatographic separation revealed the presence of isobaric molecules. Using ionic liquid based stationary phases, the selectivity of chromatographic elution changed significantly compared to regular reversed-phase chemistries. With their multiple interaction types, these novel columns were able to separate the complex mixture in a more systematic manner, more suitable to analytical and preparative separations. In general, most degradation products were classified as low oxygen-content lasses (O/C < 0.3). Therefore, subsequent reductive, deoxygenation processes of the regular common cleavage were assumed as degradation mechanisms. The enhanced mass defect filters provided a rapid overview of the formed compound classes on a macro-level; they also revealed homologous relationships based on lignin specific monomeric units (p-coumaryl, coniferyl and sinapyl) and homologous series differing in the number CH2 units at in the micro level. In conclusion, LC-HRMS readily enabled compound class assignment as well as precise identification of specific breakdown products.

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Fully automated structure verification of organic compounds by combining high resolution accurate MS with NMR data analysis

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Keywords: structure verification, synthetic chemistry, high resolution accurete mass, NMR

Einleitung

One of the bottlenecks during the drug development process in pharmaceutical industry is to rapidly verify structures. This basic task frequently involves MS/NMR experts. Here we describe a new approach which automates structure verification for (synthetic) chemists. This greatly reduces the number of cases that have to be dealt with by the "expert". Verification results, for the user, are visualized with a red/yellow/green classification. The chemist obtains this single combined classification with reject option for NMR and MS data. Further visual inspection of the data is possible but not mandatory.

Experimenteller Teil

The workflow for MS analysis includes automatic recalibration, search for target compounds, extracted ion chromatogram (XIC) calculation, masking of saturation effects, and the validation of high resolution accurate mass data as well as isotopic distributions. A robust evaluation of the statistics over individual mass spectra derived from XICs is part of the novel isotope pattern validation procedure. The compositional nature of the isotopic data is taken into account [1]. The sensitivity/specificity-tradeoff and rejection criteria for the classification are configurable. The MS analysis result is combined with an automatic NMR structure verification. Here, we focus on the MS processing method.

Ergebnisse und Diskussion

96 commercially available standards [2] were split and measured in 2 different labs (LCMS and NMR, respectively). For LCMS a UHPLC (RSLC, Dionex) was coupled to a QTOF (Compact, Bruker Daltonics). To each analysis an external calibrant was added via a switching valve. For NMR data 1H and HSQC spectra were recorded using a 400 MHz NMR spectrometer (AVIIIHD 400MHz, Bruker BioSpin). We validated the MS data processing result using receiver-operating characteristics [3]. To test the robustness of the algorithms five decoy formulas were analyzed against each of the 96 samples. The five decoy formulas are isobaric, have similar elements, and double bond equivalents to the 96 reference formulae. The test cases were classified using a range of thresholds for the intensity score. Rejects were counted as negative classification. In this evaluation, the molecular formula is classified by MS, but not the structure. The evaluation of these data with respect to the target molecular formula using a medium-conservative sensitivity/specificity-tradeoff results in a 1% false positive rate (FPR) at 89.6% true positive rate (TPR). A very conservative setting results in a 0% FPR at 56% TPR. When favoring high sensitivity, the classification still achieves a 4% FPR at 99% TPR. It's important to also recognize that the accurate mass of the MS will also rule out many false positives in advance.

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Multivariate statistics applied to MALDI-TOF MS data of pollen samples

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Keywords: Pollen, Datenanalyse, Hauptkomponentenanalyse

Einleitung
Every spring annual pollen grain monitoring begins anew to provide a pollen-screening information network for persons suffering from an allergy. The current characterization and identification of pollen is a time-consuming task that mainly relies on microscopic determination of the genus-specific pollen morphology.

A variety of new analytical approaches have been proposed in order to develop fast and reliable pollen identification using specific molecular information. Recently matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was initially applied for the rapid investigation of such complex biological samples. The combination of the obtained patterns of pollen mass spectra and multivariate statistics provide a powerful tool for the investigation of structural correlations within mixtures.

Experimenteller Teil
Both commercially available lyophilized pollen (Sigma, Germany) and fresh pollen acquired from biological samples collected in parks and in the Botanic Garden Berlin-Dahlem in the years 2013-2015, were utilized. The samples were extracted based on a modified biotyperTM protocol (Bruker) by formic acid extraction in the gas phase and spotted with HCCA matrix. To achieve this we introduce a newly developed MALDI target, with micrometer sample spots sizes. This enables the analysis of single pollen grains by MADLI-TOF MS (Autoflex III, Bruker) in order to identify taxonomic differences and relations. The spectral data were investigated by principal component analysis (PCA).

Ergebnisse und Diskussion
The applicability of MALDI-TOF MS for the classification of pollen from the plant orders Fagales and Coniferales according to their taxonomic relationships was proven by our group in a previous study [1]. Specifically, chemical differences in the mass spectra at the levels of plant order, genus and in many cases even of species could be identified [1]. Our data also show that MALDI-TOF MS of pollen can provide additional information about sugars, proteins and glycoproteins [2].

Based on these results, further investigations have been undertaken to optimize sample preparation for the classification of single pollen grains especially in mixtures. A novel MALDI-target design was developed to enhance the phenotypic information of pollens in their mass spectra. The results can be used to improve the reconstruction of taxonomic relations between species in various mixtures and might be useful for the development of a fast routine method to identify pollen based on mass spectrometry.

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Workflows for Automated Batch Analysis and Visualization of MALDI Mass Spectrometry Data

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Keywords: KNIME, mMass, MALDI, ISD, Mass-Spectrometic-Data

Einleitung

While tools for the automated analysis of MS and LC-MS/MS data are continuously improving, it is still often the case that at the end of an experiment, the mass spectrometrist needs to spend time carefully examining individual spectra.[1] Software is typically provided by the instrument vendors and is usually monolithic and difficult to adapt to rapidly evolving demands. On the other hand there are numerous academic projects for proteomics data analysis. Openly available tools to process batch MALDI mass spectrometry data is still missing. Inspired by the integration of OpenMS[2] into the workflow system "KNIME", this work shows how this gap is filled by integrating mMass into KNIME.

Experimenteller Teil

Different workflows were designed and tested:

Quantification workflow: Yeast Alcohol Dehydrogenase (Digestion Standard, Waters) with internal Standard ([Glu1]-Fibrinopeptide B human, Sigma Aldrich); Instrument: AB SCIEX TOF/TOF 4800

Identification workflow: Reduced (Dithiothreitol) and alkylated (Iodacetamide) Bovine Serum Albumin (Sigma Aldrich), Instrument: MALDI-LTQ-Orbitrap XL

MALDI-ISD workflow: Cytochrome C, 1,5-Diaminonaphthalene (Sigma Aldrich), Instrument: MALDI-LTQ-Orbitrap XL

Ergebnisse und Diskussion

Integrating mMass[3] tools into KNIME enables the user to combine automated data processing of different Open Spectrum files (signal processing, identification) with KNIME`s data mining and visualization capabilities in a single workflow. A KNIME workflow is composed of multiple nodes that are connected by ports. In spite of input and output nodes, each node receives data from the nodes, it is connected to, processes the information and finally delivers through its output port to the next node. To further structure workflows, KNIME provides so called Meta-nodes to group a collection. The integration of mMass into KNIME was possible based on nodes which interface with the scripting language Python. mMass itself is written in Python, thus the import as a module with all the classes and functions into a KNIME workflow is realized by this nodes. Moreover the functions to process raw MALDI mass spectrometry data were extended by tools enabling processing of MALDI-ISD data and performing quantifications by a relative standard. It can be summarized, that through the combination of mMass and KNIME numerous tools are accessible in a user-friendly workflow. Workflows for the analysis of MS data can easily be created, tested and shared with collaborators and used in high-throughput scenarios.

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Detektion Peroxid-basierter Sprengstoffe und strukturell verwandter Verbindungen mittels dielektrisch behinderter Entladungsionisations-Massenspektrometrie (DBDI-MS)

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Keywords: DBDI, Peroxid, Ambient-MS, Sprengstoff, TATP

Einleitung

Triacetontriperoxid (TATP) ist ein Peroxid-basierter Initialsprengstoff, der wegen seiner Synthese aus einfach verfügbaren Vorläufern vor allem in improvisierten Sprengvorrichtungen Anwendung findet. Seine hohe Empfindlichkeit gegenüber Stößen, Wärme und Reibung machen die Handhabung in größeren Mengen lebensgefährlich. Die Analyse mit klassischen Detektionsmethoden, wie UV/Vis, Fluoreszenz, ESI/APCI-MS, GC-FID/MS und Anderen ist aufgrund der Struktur und Labilität schwierig. Zur Desorption, Ionisation und Detektion von TATP und anderen Peroxiden von Oberflächen wurde die dielektrisch behinderte Entladungsionisations-Massenspektrometrie (DBDI-MS), welche auf einem Niedriegtemperaturheliumplasma basiert, eingesetzt.

Experimenteller Teil

Lösungen der untersuchten Peroxide, unter anderem TATP sowie Dibenzoylperoxid (DBPO), wurden auf verschiedene Oberflächen aufgetragen und mit einer Ammoniumacetatlösung überschichtet. Die Zugabe von Ammoniumacetat dient hierbei der Verbesserung der erhaltenen Signalintensität. Nach der Trocknung wurden die zu analysierenden Oberflächen mit Hilfe der DBDI-MS vermessen. Deuteriertes TATP (TATP-d18) wurde zur Bestätigung der erhaltenen Signale eingesetzt. Da aufgrund der zur Handhabung notwendigen Restfeuchte des TATP das Einwiegen mit einem großen Fehler verbunden ist, wurde die quantitative Kernspinresonanzspektroskopie (qNMR) zur Bestimmung der Konzentration der TATP-d18-Lösung, welches bei der Quantifizierung von TATP mittels DBDI-MS als interner Standard dient, eingesetzt.

Ergebnisse und Diskussion

Bei der Analyse von TATP und der Zugabe von Ammoniumacetat weist das Massenspektrum ein Signal bei m/z 240 auf, welches dem Ion [M+NH4]+ entspricht. Die Zuordnung konnte durch die Analyse von deuteriertem TATP (TATP-d18, m/z 258) bestätigt werden. DBPO wird analog zu TATP als [M+NH4]+ detektiert. Eine Quantifizierung von TATP konnte mit Hilfe von TATP-d18 als internem Standard erfolgen. Mittels der dielektrisch behinderten Entladungsionisations-Massenspektrometrie können Peroxid-basierte Sprengstoffen wie TATP und strukturell verwandte Verbindungen, wie DBPO, ionisiert, detektiert und quantifiziert werden.

Referenzen

Posternummer: 167

Evidence for laser-induced redox-reactions between added trifluoroacetate salts and substrate material during polystyrene/DCTB matrix-assisted laser desorption/ionization

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Keywords: MALDI, Polystyrene, Redox-reactions, Coordination chemistry, HSAB

Einleitung

Polymers, such as polystyrene, have been successfully analyzed with matrix-assisted laser desorption/ionization (MALDI) through the addition of e.g. copper or silver salts. This method is often used to establish the polydispersity index of polymer blends. However, the mechanism of cation addition and the possible interactions between the added salts and the chosen target material are still points of interest. Therefore, the addition of several trifluoroacetate salts to a mixture of polystyrene and matrix on a range of different target plate materials was systematically investigated, revealing several new interesting aspects of MALDI.

Experimenteller Teil

Polystyrene (Mw 1,920 Da) was mixed with a range of trifluoroacetate salts (Li, Na, K, Cs, Ba, Cr, Pd, Cu, Ag, Zn, Al and In, as well as trifluoroacetic acid) and analyzed with MALDI using 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as matrix on different target plate materials (chrome, copper, silver, gold, Ti90/Al6/V4, Inconel® 625, Zinc and stainless steel) to evaluate the occurrence of redox-reactions.

Polystyrene/salt/matrix solutions were deposited through pneumatic-assisted spraying on microscope slide-shaped target plate insets of varying material, which, secured with copper tape, fitted a milled out structure from the original target plate. Spectra, obtained on a Bruker Autoflex I MALDI-Time-of-Flight mass spectrometer, were processed with MATLAB to obtain polystyrene- and matrix-adduct ion signal intensities for direct comparison between chosen conditions.

Ergebnisse und Diskussion

The resulting spectra shed light on the MALDI adduct formation process and the cation-polystyrene interactions. It was found that the following cation-polystyrene adducts were formed on stainless steel: Al, Li, Na, Cu and Ag, where the yield was found to depend on the sample layer thickness and possibly the cation's ability to form a complex with either one or two of polystyrene's phenyl rings, based on the ligand-field and the valence bond theory. With the exception of Al, these salts also formed adducts and in case of Cu and Ag also sandwich adducts with DCTB. Some alkali salts (e.g. potassium) formed clusters rather than interacting with polystyrene or DCTB, which can be explained with the HSAB theory. Application of TFA salts on a copper surface led to copper cation formation, resulting in DCTB and polystyrene copper-adduct formation. The same effect occurred for silver substrate. In the absence of copper or silver salts, it is therefore still possible to form their respective adducts by choosing the proper alternative salt (e.g. Li, Cs, Ba, Cr) in combination with either a silver or a copper substrate surface. Incubation tests with copper beads in various salt solutions, before matrix and polystyrene addition, support that copper ions are not generated during the deposition process before the MALDI experiment is carried out, except when trifluoroacetic acid, indium and aluminium trifluoroacetate are used. For all other salts used on a copper plate, it can therefore be concluded that these copper cation forming redox-reactions are enabled by the input of laser photon energy. Furthermore, it was discovered that copper beads can successfully sequester polystyrene from the sample mixture, indicating the strong bonding of polystyrene to the copper surface. These findings support that the redox-reactions occur (almost) instantaneously with laser pulse impact at the sample-coated substrate surface.

Referenzen

Laser induced plasma ion source for ambient mass spectrometry

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Keywords: laser induced plasma, high repetition rate laser, ambient mass spectrometry, emission spectroscopy

Einleitung

Directly after the invention of the laser, laser induced mass spectrometry was employed. At excessive irradiation microplasmas were observed as a potential channel for ion formation. However, plasma ignition in vacuum led to undesired distortions and was discarded as ion source.

Effective cooling under ambient conditions resulted in more controllable plasmas and development of laser induced breakdown spectroscopy (LIBS). However, little effort has been made to combine LIBS and ambient MS, since these plasmas only provide microsecond lifetimes, reducing the duty cycle. After these microseconds, recombination yields uncharged and newly bound species, making them inaccessible for MS.

The combination of high repetition rate lasers together with growing knowledge about manipulation of charged species at atmospheric pressures allow overcoming these obstacles.

Experimenteller Teil

A laser induced plasma was ignited under ambient conditions by focusing the output of a frequency doubled high repetition rate diode pumped solid state Nd:YVO4 laser (λ = 1064nm + 532 nm, 1-500 kHz) through an aspherical lens (NA = 0.50) with a focal length of 8 mm. The plasma was positioned in the vicinity of a modified orthogonal time-of-flight mass spectrometer.

Additional gas support was enabled by placing the glass tube of a DBD probe co-axially in front of the inlet. The flow was monitored and adjusted in the range of 0 L/min to 2.0 L/min by a mass flow controller.

Further insights into the plasma chemistry were obtained by performing VUV and VIS emission spectroscopy experiments.

Ergebnisse und Diskussion

Current findings reveal that the ion source could be a promising ambient sampling scheme. Upon plasma ignition immediate signal response was observed.

The ion pattern resembles those detected for other ambient plasma-based ion sources, such as DART, helium-driven DBD or the microplasma source. The mass spectrum is governed by a series of protonated water clusters up to higher masses of 1 kDa, with maximum signal intensities for [(H2O)n+H]+ with n = 2, 3 and 4, accompanied by additional reactive species, such as NH4+ with minor abundance. In presence of a molecule with a higher proton affinity, these water clusters undergo proton transfer reactions yielding protonated molecular ions.

After demonstration of the general concept, the performance was optimized, as several parameters showed effects on the sensitivity, robustness and stability of the ion signal. Among these the repetition rate of the laser, the laser power, the ion guide settings of the mass analyzer and the addition of different gases (air, N2, Ar, He) was investigated to yield the highest abundance of the above mentioned water clusters. The promoting role of applied gas flow could be observed to be either an enhancement of the fluiddynamic flow, as well as an increase in plasma formation due to different ionization potentials. Also the number of internal degrees of freedom (and thus the gases heat capacity cP) could be observed to play a role.

For analysis of test compounds, the substances were placed in a gas-tight flask and were passed by a gentle gas stream, which is directed via the DBD glass tube into the plasma region.

Since formation of reactive species was found to be highly effective, but further understanding of the participating mechanisms and reactions in the plasma is desired for maximum ionization, comprehensive emission spectroscopy experiments were conducted in the VUV and Vis-range.

Referenzen

Reactive low temperature plasma (LTP) mass spectrometry for effective determination of organic UV filters and parabens in cosmetics

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Keywords: reactive low temperature plasma; ambient mass spectrometry; cosmetic analysis

Einleitung

Organic UV filters are important ingredients in sunscreen products to protect the skin of the user from sunburn. Parabens are commonly added into cosmetics as preservatives. However, both additives have been reported to potentially threaten human health. Up to now, the mostly used techniques to monitor such components in cosmetics are GC-MS and HPLC-MS. These chromatography-based methods are precise but labor- and time-consuming. Meanwhile, ambient mass spectrometry (MS) techniques such as low temperature plasma (LTP) –MS perform impressively in in-situ analysis, but they still show limits in direct determination of nonvolatile compounds. Therefore, a reactive LTP-MS method is introduced here for the rapid and sensitive detection of parabens and organic UV filters with low volatility in cosmetics.

Experimenteller Teil

A home-built LTP source1,2 combined with a linear ion trap / Fourier transform ion cyclotron resonance hybrid mass spectrometer (LTQ FT Ultra, Thermo Fisher Scientific, Bremen) was used for analysis of the samples. Helium (99.9999%) served as the main working gas at a flow rate of 1 L/min. The plasma was generated by dielectric barrier discharge3. Standards of the UV filters and parabens were dissolved in methanol to prepare standard solutions. Then 1 µL of the standard solution was loaded onto a stainless mesh strip and was analyzed by LTP MS in transmission geometry. Reactive reagents were mixed with the sample solutions or added into the working gas system by an injection pump during mass analysis.

Ergebnisse und Diskussion

In order to detect both classes of additives efficiently, several chemicals, including ammonia, ammonium acetate/formate/chloride, and methylamine were considered as reactive reagents. The reactive reagents were firstly mixed with the sample solutions and analyzed directly with LTP-MS. The mass spectra of the mixed sample and the pure sample solutions were compared to check if the reaction happened efficiently. Methylamine was found to react with the sample quickly and to produce strong MS signals of the reaction product [M+NH2CH3+H]+. Formation of the imine adducts benefits the detection of UV filters through a prominent increase of the desorption efficiency, while it enhances the analytical performance of parabens in the positive ion mode by offering an easier ionization pathway than the protonation of the acidic analytes. Other reagents tested, such as the ammonium salts and ammonia, however, showed no or less reactivity than methylamine. In the next step methylamine was added into the working gas system through an injection pump to create a stable reaction pathway. In preliminary experiments, compared with direct LTP-MS detection, reactive LTP-MS was found to enable the efficient detection of non-volatile UV filters such as avobenzone without extra heating, and to increase the detection sensitivity of UV filters and parabens. In the future, the influence of the concentration of methylamine on the MS signal will be evaluated, and quantitative analysis of the sample with the imine-induced reactive LTP will be studied. Furthermore, this method will be applied in the analysis of real cosmetic products.

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Reproduzierbarkeit und Grundlagen der papierbasierten Atmosphärendruck Massenspektrometrie

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Keywords: Atmosphärendruck Ionisation, Papierbasierte Massenspektrometrie, Instrumentelle Entwicklung

Einleitung
Die papierbasierte Massenspektrometrie (PB MS) ist ein vielversprechendes Ionisationsverfahren unter atmosphärischen Bedingungen, welches seit 2010[1][2] rege Forschungsinteressen erfährt. Die großen Vorteile der PB MS sind die Einfachheit der Umsetzung des Prinzips der Methode, auch bei komplexen Matrizes, und die geringen Kosten des verwendeten Materials.

Das Hauptaugenmerk der meisten Publikationen auf diesem Gebiet liegt auf den erhaltenen positiven Ergebnissen. Dazu zählen beispielsweise die Quantifizierung verschiedener Drogen in getrockneten Bluttropfen[1] und verschiedene Möglichkeiten der Probennahme, wie das Wischen über eine Oberfläche.[2]

Für eine Weiterentwicklung in diesem Bereich sind darüber hinaus auch kritische Publikationen, die sich mit den Grenzen der Methode beschäftigen, nötig. Das Ziel dieser Arbeit liegt im Aufzeigen der Herausforderungen dieser Technik und der Bewältigung von ihren.

Experimenteller Teil
Die verwendeten Papierspitzen wurden in Form von gleichschenkligen Dreiecken verwendet. Es erfolgte eine koaxiale und zentrale Justierung der Spitzen in ca. 5 mm Entfernung zum Einlass des Massenspektrometers. Es wurden 10 µL Argininlösungen unterschiedlicher Lösungszusammensetzung (10 mM; Wasser; Wasser/Methanol, 1:1, v/v; Wasser/Methanol/Essigsäure, 1:1:0.01, v/v) auf den freien Bereich der trockenen Papierspitze pipettiert. Im nächsten Schritt wird Spannung angelegt und eine MS (API HTOF-MS) Messung bis m/z 800 durchgeführt. Die Spannung betrug, wenn nicht anders notiert, 3 kV. Es wurde ausschließlich der positive Ionenmodus verwendet.
Für eine Korrelation der erhaltenen Ionensignale zur mikroskopischen Beschaffenheit der Papierspitzen wurden zusätzlich optische Mikroskopbilder mit einem Ramanmikroskop) und einem 10x-Objektiv aufgenommen.

Ergebnisse und Diskussion
Diese Arbeit enthält systematische Untersuchungen zu verschiedenen Einflussfaktoren der PB MS. Im Vordergrund standen dabei Erkenntnisse zur Reproduzierbarkeit und Standardisierung der Methode. Durch den Vergleich der Zeitverläufe des Ionenstroms und aus den daraus resultierenden Spektren, selbst unter gleichen Bedingungen, zeigte sich, dass die Reproduzierbarkeit einzelner Messverläufe und der daraus erhaltenen Ergebnissen verbessert werden muss. Dies betrifft sowohl Messungen unter gleichen Rahmenbedingungen, als auch solche mit unterschiedlichen experimentellen Aufbauten. Die Reproduzierbarkeit ist somit die aktuelle Herausforderung der PB MS Forschung, die einer universelleren Verwendung als Ionisierungsmethode der Wahl im Wege steht. Die Beschaffenheit, zufällige Anordnung und Ausrichtung der Papierfasern, genauer die Morphologie des Materials selbst spielt dabei eine erhebliche Rolle.[3] Darüber hinaus stützen die in dieser Arbeit erhaltenen Daten die Vermutung, dass der zu Grunde liegende Mechanismus der PB MS auf den Antenneneffekten jener Faserenden beruht.[4] Die zufällige Verteilung der Faserenden ist ein Unsicherheitsfaktor, der durch die Verwendung strukturmodifizierter Papiere ausgeglichen werden soll. Der Einsatz dieser Papierarten führt in der Regel zu verringerten Signalintensitäten. Die Unterschiede zwischen den Papieren ergeben sich neben der unterschiedlichen chemischen Komposition der Fasern, die beispielsweise die Hydrophobizität beeinflusst, auch aus den morphologischen Details der jeweiligen Papierart. Diese umfassen neben den oben genannten Einflussgrößen auch die makroskopische Dicke und Starrheit des Papieres selbst.
Es ist durch die Vielzahl der voneinander unabhängigen Einflussgrößen schwierig, allgemein gültige und optimale Konditionen für die PB MS zu entwickeln. Jedoch konnten Einblicke in den Spray- und Ionisationsvorgang gewonnen werden. Als Konsequenz dieser Erkenntnisse konnten verschiedene Möglichkeiten der Signalverstärkung und Verbesserung der Reproduzierbarkeit getestet werden, die vielversprechende Ergebnisse lieferten, jedoch weiterer Optimierung bedürfen.

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