Water and Metabolite Diffusion in Tissue

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OVERVIEW In Vivo MR Spectroscopy at large diffusion weighting can specifically look at intracellular components of tissue that are characterized by restricted diffusion and a small apparent diffusion coefficient. Beyond water, ^{1}H MRS of metabolites at large diffusion weighting proved to be very useful to separate extra- and intracellular contributions of metabolites in the brain. Further application of diffusion-weighted MRS with other nuclei than ^{1}H, e.g., ^{31}P, ^{19}F, ^{7}Li, ^{13}C, can be utilized to answer specific questions tied to various animal models of disease like ischemia, excitotoxicity, tumor or hydrocephalus (1). Such diffusion-weighted spectroscopy is a powerful tool, using information tied to different compartments, different metabolites, and different tracers.

^{1}H Spectroscopy at Very Large Diffusion Weighting

Water Signal - Multiple components and Non-Exponentiality

Many different approaches and models have been studied to date to understand the biophysical basis of the changes of the apparent diffusion coefficient (ADC) in tissue. Experiments in isolated cells, nerves or in vitro cell preparations are useful to separate the intracellular contributions. Utilizing the different diffusion properties in the different cellular structures, i.e., restricted vs. hindered diffusion, the strength of the diffusion weighting itself can provide a separation of signal belonging to different cellular 'compartments', e.g. extra- vs. intracellular. Such diffusion MRS has been used for a decade to look at separated intracellular signals at large diffusion weighting (2). In a model with cell threads, providing a clean 'intracellular' compartment, fast changes can be observed within minutes of induction of osmotic stress, which causes cell swelling (Fig. 1a) (3). It was shown that D^{app} of the intracellular space is on the order of 0.1 μm^2/ms, one to two magnitudes lower than the free diffusion of water. Similar fast changes were observed during global ischemia in rat brain in vivo; starting 40 s after cardiac arrest, the water signal at large diffusion weighting increases within 60 s (Fig. 1b) (4).

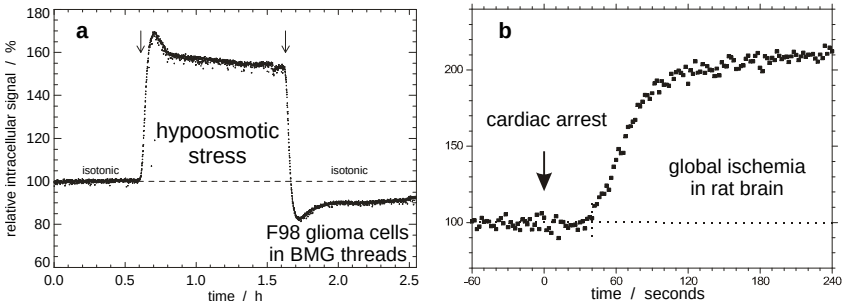

Figure 1: Monitoring of fast signal changes by ^{1}H MRS of the water signal at large diffusion weighting. (a) swelling of glioma cells upon osmotic stress at b = 15 ms/μm^2. (b) Global ischemia in rat brain. 40 s after cardiac arrest, the DW signal increased fast within 60 s (b = 13 ms/μm^2, t_{diff} = 32 ms). Refs (3,4).

The signal attenuation of water can be studied in vivo using large diffusion times and weightings (Fig 2a). The common analysis to fit the signal attenuation to a multi-exponential decay imposes a prior knowledge of an exponential model. The outcome of two, three, or more components implicitly suggests that these signal components can be interpreted as biological "compartments",

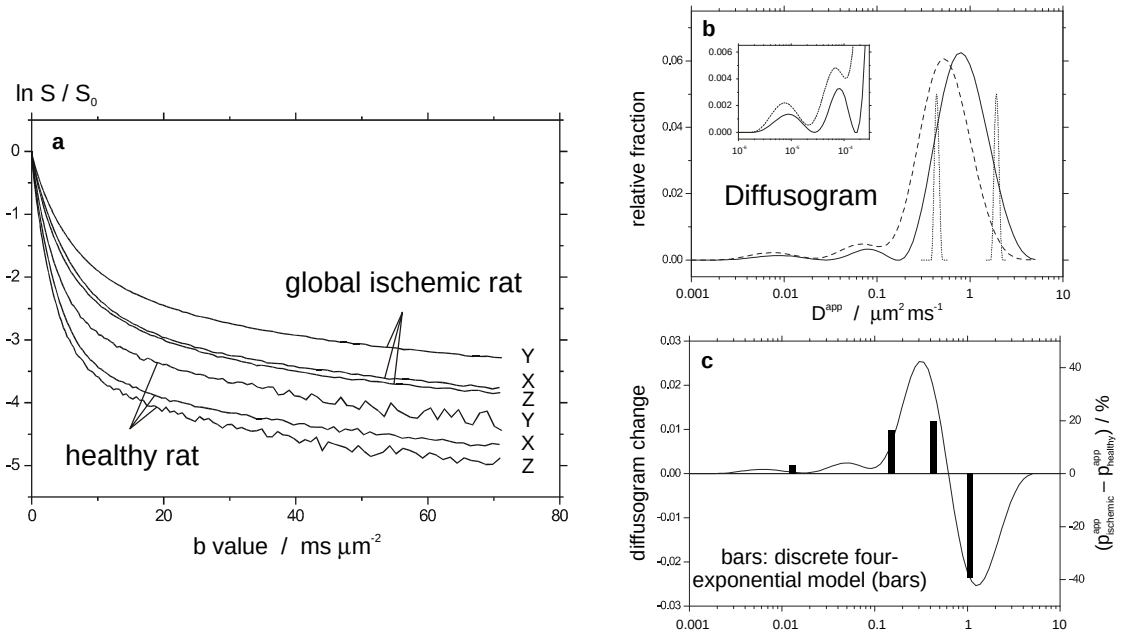

Figure 2: (a) Water signal attenuation in rat brain *in vivo* and *ex vivo* (within one hour after cardiac arrest) in x, y, z-direction (t_{diff} = 115 ms). (b) Histogram plot of the relative fractions of quasi-continuously distributed diffusion coefficients D^{app}, showing a broad distribution of D^{app} with three peaks (normal (solid), ischemic (dashed), phantom experiments (dotted sharp peaks). (c) Changes of diffusion components (volume fractions) after ischemia using the diffusogram (solid line) and a discrete four-exponential model (bars). Ref. (4).

which should be done very carefully, since the results of the fit depends on the experimental parameters (like b range or diffusion time) and actually has mostly a statistical meaning. If one uses a different analysis, like a Laplace Transform, one finds a widely distributed range of D^{app} in a Diffusogram. In the case of our experimental setting, we found three wide humps at 1, 0.1 and 0.01 µm^2/ms (Fig. 2b) (4). (Note that the monoexponential signal decay of a phantom gives a relatively sharp peak !).

The interpretation is more complicated when comparing the normal and ischemic case: one finds a shift in the D^{app} of the Diffusogram (like an ADC decrease), which in contrast can be interpreted as one decreasing ('extracellular') and one increasing ('intracellular') component (Fig 2c). The latter is found when a conventional four-exponential fit is used (bars in Fig. 2).

Cerebral metabolites – Extracellular contributions

Similar to the pure "intracellular" cell models, one can use intracellular markers, like NAA, to study the properties of intracellular diffusion (5). Using single-voxel MRS, diffusion properties of more than 10 cerebral metabolites can be studied (Fig. 3, 4) (6,7). Using high magnetic field (9.4 Tesla), even glucose signal at very large diffusion weighting can be detected (Fig. 4b). Multi-exponential decays were found for all metabolites, including glucose (Glc) and lactate (Lac).

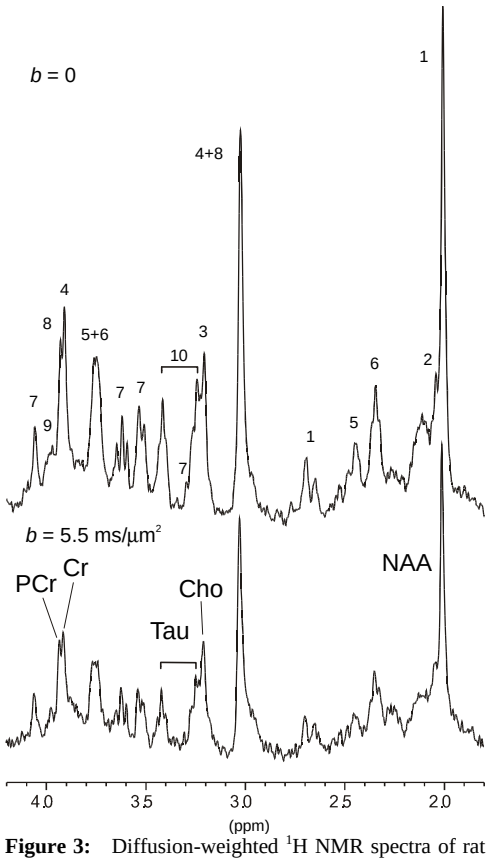

Figure 3: Diffusion-weighted ^{1}H NMR spectra of rat brain *in vivo* (t_{diff} = 31 ms). The attenuation S/S_0 of 40-50% corresponds to a D^{app} of 0.09–0.12 µm^2/ms. NAA (1), NAAG (2), Cho (3), Cr (4), Gln (5), Glu (6), Ins (7), PCr (8), PE (9), Tau (10). Ref. (6)

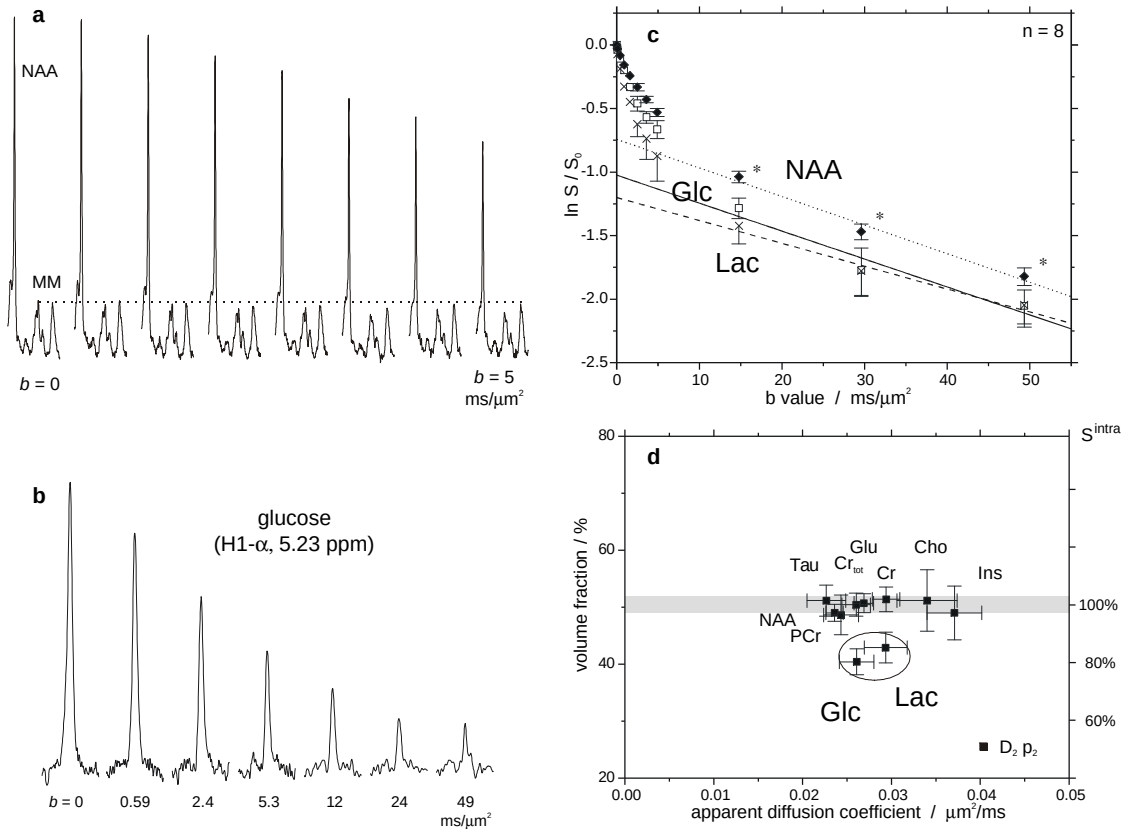

Figure 4: ^{1}H MRS at very large diffusion-weighting. (a) NAA, macromolecules (2.2-0.7 ppm). (b) H1-α-glucose, detectable at large b. (c) Bi-exponential diffusion decay (t_D = 119 ms, TE = 22 ms, TR = 4 s, n = 12). The intercept of the second component of Glc and Lac is significantly smaller compared to NAA and the other metabolites. (d) Volume fractions of the second component at large b are very similar for most metabolites and taken as internal reference (100%). The relative intracellular signal S^{intra} of Glc and Lac was ~80%. Ref. (6)

The data in Fig. 4b and 4c prove that a significant fraction of cerebral glucose *in vivo* is located in intracellular compartments and is being restricted in diffusion. Most other metabolites had very similar D^{app} at small and large b values. Glc and Lac revealed a larger D_1^{app} at small b (ADC) and a significantly lower intercept of the second component at large b (p_2^{app}). The volume fractions p_2^{app} of the slowly diffusing signal components are shown in Fig. 4d. Taking NAA as a reference for a "purely" intracellular metabolite (S^{intra} = 100%), the calculated relative intracellular signal of Glc and Lac was found to be ~80% (right scale). In other words, this is consistent with a ~20% extracellular signal, which contributes and increases the ADC at small diffusion weighting.

Diffusion-Weighted ^{13}C Spectroscopy

Other interesting applications of MRS at large diffusion weighting use labeled metabolites in combination with MRS of other nuclei than ^{1}H, e.g., ^{31}P, ^{19}F, ^{7}Li, ^{13}C (1). Here, we show the feasibility of diffusion-weighted {^{1}H-^{13}C} spectroscopy *in vivo* to examine the compartmentation of ^{13}C-labelled, metabolically-active lactate in a rat tumor model (8). To improve sensitivity, indirect ^{1}H detection of ^{13}C label with the ACED STEAM sequence (9) was used (t_D = 120 ms).

Phantom experiments in a 20 mM [3-^{13}C] lactate solution were performed at different diffusion weightings (Fig. 5a-d). Carbon-13 signals were inverted with an editing pulse during TM (Fig. 5a). Subtraction of alternately recorded spectra (Fig. 5a and 5b) yielded proton signals of lactate connected to [3-^{13}C]. With decoupling in the ^{13}C channel, single resonance proton spectra of lactate were obtained representing total lactate concentration (^{12}C+^{13}C, Fig. 5c) or solely ^{13}C lactate (Fig. 5d).

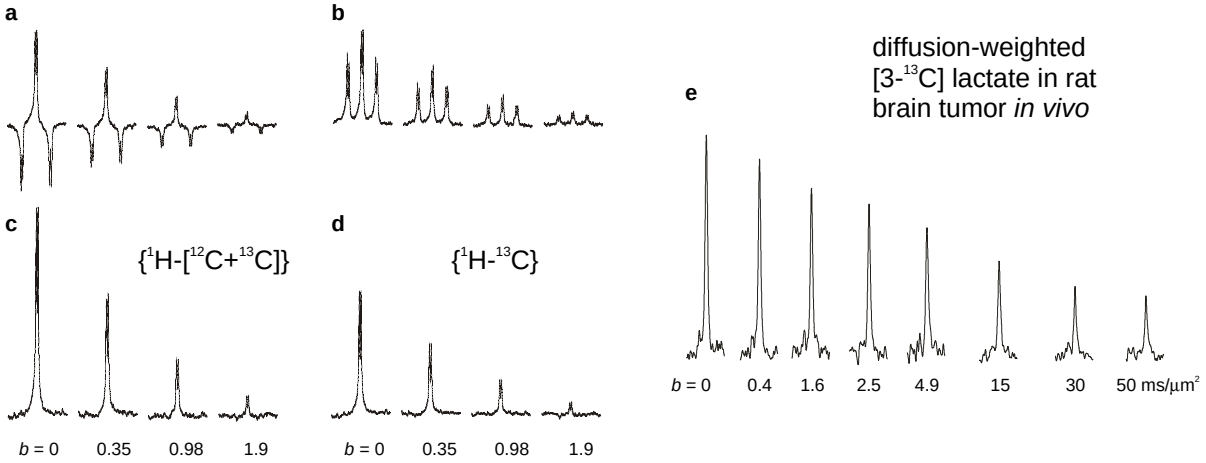

Figure 5: Diffusion-weighted ^{13}C MRS acquired with ACED-STEAM. (a)-(d) Phantom experiments, [3-13C] lactate with the 13C signals inverted each other scan. (d) Subtraction and decoupling results in the carbon-edited series. (e) in vivo detection of diffusion-weighted 13C lactate signal in rat brain tumor. Significant signal at large b is consistent with a large intracellular volume fraction. Ref. (8)

100% [1-^{13}C] enriched glucose was continuously infused i.v., which was metabolized to [3-^{13}C] lactate in the tumor. In the rat glioma tumor, signal from [3-^{13}C] lactate could be observed up to very large diffusion weighting (Fig. 5d). ^{13}C fractional enrichment of lactate was about 70% after one hour of [1-^{13}C] glucose infusion. The apparent diffusion coefficient was ~0.10 $\mu m^2/ms$ in the b range of 5 $ms/\mu m^2$. At very large *b* values, a bi-exponential signal decay was found with $D_{1,2}^{app}$ = (0.2, 0.01) $\mu m^2/ms$ and volume fractions $p_1:p_2 \sim 0.5:0.5$.

In several respects the use of [1-^{13}C] glucose infusion is advantageous for the detection of [3-^{13}C] lactate in a tumor model. Only signal from metabolically-active tissue (metabolizing glucose to lactate) is observed giving an intrinsic selectivity and localization in the tumor. The editing technique eliminates unwanted lipid signals often found concomitant with the lactate signal in ^1H NMR spectra of tumors. The high concentration and fractional enrichment of lactate is advantageous for spectroscopic methods. Multi-exponential diffusion attenuation can be used to distinguish intra- from extracellular signals due to a different diffusion characteristic (restricted and hindered diffusion). Based on a comparison with metabolites known to be intracellular, the intra/ extracellular compartmentation of lactate in tumor appears similar to a previous study in normal rat brain (6).

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