Development of a lectin-affinity chromatography step for the downstream processing of influenza virus vaccines

M.W. Wolff[1], J. Salaklang[2], L. Opitz[1], J. Schwarzer[1], E. Rapp[1], B. Kalbfuss[1], U. Reichl[1,3]

[1] Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany; [2] Department of Chemical Engineering, University of Applied Science, Münster, Germany; [3] Department of Bioprocess Engineering, Otto-von-Guericke-Universität, Magdeburg, Germany

Influenza remains due to its annual death rate and potential to cause pandemics a major public health concern. Efforts to control the annual spread of influenza have centered on prophylactic vaccinations. Human influenza vaccines are traditionally produced in embryonated hen's eggs. However, major constraints with this method, e.g. allergic reactions induced by egg proteins and lack of scalability have lead to the development of cell culture based production processes. In recent years, several continuous cell lines such as the Madin Darby canine kidney (MDCK) or the African green monkey kidney Vero cells have been successfully established for the production of influenza vaccines in cell culture. These processes require the modification of existing but also the development of new downstream strategies to account for the changed upstream technology. Downstream processing of biological products is conventionally subdivided into three steps: capture or concentration, separation or fractionation and polishing. The capture step is commonly the most expensive unit operation. Hence, the efficiency of this step has a large impact on the total process economics.

The presented study focuses on the development of a proficient capture step based on lectin-affinity chromatography. Lectins are a class of carbohydrate specific proteins of non-immune origin that have a selective affinity for a carbohydrate or a group of carbohydrates. Immobilized lectins have been used successfully for many years to separate and isolate glycoconjugates, polysaccharides, soluble cell components, and cells containing glycoproteins with specific carbohydrate structures on its surface. The influenza A virus contains two spike glycoproteins on its surface: hemagglutinin (HA) and neuraminidase (NA). HA is the most abundant surface protein. It is a trimeric glycoprotein containing per subunit 3 to 9 *N*-linked glycosylation sites depending on the viral strain. Here the influenza A/PR/8/34 virus has been selected as a model. The HA molecule of this particular virus contains according to the NetNGlyc 1.0 Server prediction six glycosylation sites. Detailed analysis of these sites and their individual glycan structures are presently performed. Based on preliminary structural glycan analysis studies and literature data several HA-binding lectins are selected for a pre-screening via lectin-blots. The most promising lectin-blot results are obtained from lectins specific for terminal galactose e.g. Erythrina cristagalli (ECL), Arachis hypogaea (PNA). Lectins, by which lectin-blot analysis suggests an interaction with viral membrane proteins, are currently screened for their suitability as an affinity matrix ligand. Therefore, centrifuged cultivation broths of influenza A/PR/8/34 virus infected MDCK cells are applied to various agarose-immobilized lectins. Components interfering with the immobilized lectins are selectively adsorbed. Non or weak binding components are washed from the column. Subsequently, bound components are dissociated from the lectin by competitive elution with suitable hapten carbohydrates. This fraction contains the influenza virus

particles and virally encoded membrane proteins, which have to be further processed for vaccine manufacturing. The extend of the subsequent purification depends on the specificity of the lectin binding to virally encoded surface proteins. Lectins with weak or no interaction with host cell proteins or medium components and strong interaction with viral membrane glycoproteins represent a powerful tool to concentrate and purify viral surface proteins from contaminating nucleic acids, medium components, and non-virally encoded host cell proteins.