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Development and characterization of affinity and pseudo-affinity based methods for cell culture-derived influenza virus capturing

Dissertation – Lars Opitz

Influenza is one of the most worldwide-spread diseases, which infects several million people every year. Besides antiviral medical treatments, prophylactic vaccinations are crucial for controlling seasonal influenza epidemics. Hence, every year large amounts of vaccine doses have to be produced. Conventionally, embryonated chicken eggs are used for human influenza vaccine production. However, this production process has only a limited scalability. In addition, these vaccines contain egg-derived proteins, which may cause allergic reactions. Hence, cell culture-based vaccine production processes have been developed, which require an adapted downstream processing strategy for virus purification.

The scope of this dissertation was the development of affinity- as well as pseudo-affinity-based chromatographic unit operations for the downstream processing of cell culture-derived influenza virus particles. Therefore, two major approaches were investigated: lectin-based affinity chromatography and sulphated cellulose matrices-based pseudo-affinity chromatography. In both fields membrane- and bead-based techniques were considered and compared.

Lectin affinity chromatography was developed first for the Madin Darby canine kidney (MDCK) cell culture-derived human influenza virus A/Puerto Rico/8/34 (H1N1). This dissertation showed that the α1,3-galactose-specific *Euonymus europaeus* lectin (EEL), immobilized on polymer beads, is a suitable ligand for affinity purification of glycosylated viral envelope proteins such as the hemagglutinin. The dissociation of the virus-ligand complex was done by competitive elution with lactose. More than 90% of the influenza virus hemagglutination activity was recovered in the product fraction, while the majority of host cell proteins and nucleic acids were depleted.

For chromatography, matrix selection plays an important role regarding purification efficiency. Therefore, different matrices for EEL as ligand were screened. These supports included stabilized reinforced cellulose membranes, polymer and porous glass particles, cellulose and agarose beads. Strong virus binding was achieved by EEL-modified cellulose membranes and polymer beads. Furthermore, reinforced cellulose membranes had a far better binding capacity than other tested adsorbents.

To determine the general applicability of EEL-affinity chromatography, studies were extended for two other MDCK cell-derived influenza virus strains (A/Wisconsin/67/2005 (H3N2), B/Malaysia/2506/2004). Both virus strains were captured efficiently by the ligand EEL. These results emphasized the EEL-affinity chromatography as a valuable technique for capturing MDCK cell-derived influenza virus particles. Additionally, the impact of host cells on lectin affinity chromatography has been evaluated. In contrast to findings with viruses propagated in MDCK cells, Vero cell-derived influenza virus A/Puerto Rico/8/34 bound to β1,4-galactose-specific *Erythrina christagalli* lectin (ECL) but only very limited to the α1,3-galactose-specific EEL.

The second major project part of this dissertation describes a capturing method for influenza viruses (A/Wisconsin/67/2005 (H3N2), A/Puerto Rico/8/34 (H1N1), B/Malaysia/2506/2004) using sulphated reinforced cellulose membranes. Purification efficiency with regards to viral yield as well as total protein and host cell dsDNA depletion was directly compared to commercially available cation-exchange adsorbers and to column-based Cellufine® sulphate resin. With the sulphated membranes, high product recoveries and contaminant reductions were possible. Due to a fast binding kinetic and a low back pressure, these membrane adsorbers enabled the capturing process to be operated at an increased flow rate leading to significantly enhanced productivity. Hence, sulphated membrane adsorbers are a valuable choice for industrial influenza vaccine purification processes.