Displacement Chromatography for Proteomic Applications

by

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ABSTRACT

Ion exchange displacement chromatography, chromatographic modeling, and mass spectrometry were employed to develop a new proteomics analysis platform which has the potential to achieve increased sensitivity and dynamic range for complex proteomic applications. Studies were carried out using high affinity, low molecular mass displacers for the ion exchange displacement of proteins and peptides to determine the limits of this approach. Model protein mixtures were employed to study the effects of varying displacer concentration and feed dynamic range. Results indicated that higher displacer concentrations produced elevated product concentrations at the price of reduced yields. Further, high affinity displacers employed at relatively low concentrations resulted in high resolution separations amenable to the identification of low abundance proteins. Good agreement was obtained between simulations carried out using a Steric Mass Action (SMA) based chromatographic model and the experiments performed. Parametric simulation studies were then carried out using the SMA model to investigate the concentration effects and enrichment factors observed over a wide range of conditions. Proof-of-concept column separations were carried out which illustrated that ion exchange displacement chromatography could be employed for protein pre-fractionation of complex model mixtures. This work demonstrated that displacement chromatography could be employed for trace component detection of proteins not found in feed mixtures, and that these displacement separations were amenable to subsequent mass spectrometry analysis without further processing since these displacement separations could be performed with minimal or no salt in the carrier. Displacement separations were also performed on the soluble proteins obtained from an *E. coli* cell lysate. These analyses indicated that proteins not detected in the feed mixture (due to their low concentrations) were able to be identified in the collected displacement effluent fractions. Off-line peptide fractionations using traditional and carrier displacement chromatography were also performed. These separations yielded encouraging results, both in terms of the grouping of peptides into various fractions having similar resin affinity and in terms of the concentration and enrichment of the peptides observed. Finally, a salt-free ion exchange process was developed to permit direct coupling of ion exchange displacement

separations with on-line mass spectrometry analysis. This an important result since ion exchange chromatography and mass spectrometry typically cannot be directly coupled due to the high salt concentrations required for solute elution (for elution separations) and the inability of mass spectrometers to handle this salt concentration. The results presented in this thesis demonstrate the ability of ion exchange displacement chromatography to focus trace proteins into concentrated and fractionated zones. This body of work may have significant implications for the implementation of displacement chromatography for separations where trace enrichment is critical such as proteomic applications.