High Affinity and Selective Displacers for High Resolution Protein Separations in Ion-Exchange Chromatography: Displacer Ranking, Mechanism Elucidation, and Displacer Design

by

Jia Liu

An Abstract Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the degree of

DOCTOR OF PHILOSOPHY

Major Subject: Chemical and Biological Engineering

The original of the complete thesis is on file
in the Rensselaer Polytechnic Institute Library

Examining Committee:

Prof. Steven M. Cramer, Thesis Adviser

Prof. James A. Moore, Member

Prof. Curtis M. Breneman, Member

Prof. Jonathan S. Dordick, Member

Rensselaer Polytechnic Institute
Troy, New York

November 2006
(For Graduation December 2006)

ABSTRACT

The work in this thesis focused on an investigation of the relationship between the displacers' structures and their corresponding efficacy/selectivity in ion-exchange chromatographic systems. In the cation-exchange high affinity displacer study, the displacer concentration required to displace 50 % of proteins bound in batch adsorption systems, DC-50, was employed as a means of ranking displacer affinities in the evaluation of a relatively large data set of cationic displacers with varying chemistries in parallel batch screening experiments. The resulting DC-50 data was used in concert with molecular structural information of the displacers to produce predictive quantitative structure-efficacy relationship (QSER) models that provided insight into the factors influencing displacer efficacy in cation exchange systems. In addition, a homologous series of sugar-based molecules were synthesized and evaluated by the parallel batch displacement assay as potential displacers for protein purification in anion exchange systems. The percent protein displaced was evaluated in these batch systems for two model proteins, BSA and trypsin inhibitor, as a function of displacer concentration and chemistry on a Source 15Q anion exchange resin. The results indicated that aromatic rings and structural flexibility both improved displacers' efficacies while long hydrophobic side chains impaired the displacers' efficacies. In the selective displacer study, lead compounds were selected from a commercially available database to identify potential selective displacers for a binary protein mixture in ion exchange chromatography. The batch displacement results indicated that most of these lead compounds were indeed selective for displacing ribonuclease A. In fact, one of these displacers exhibited extremely high selectivity, displacing essentially all of the ribonuclease A while displacing no α-chymotrypsinogen A at a displacer concentration of 10 mM. These results were validated under column conditions, with the ribonuclease A being displaced and the α-chymotrypsinogen A remaining in the column after the displacer breakthrough. Surface Plasmon Resonance results indicated that the protein-displacer interaction was playing an important role in the selective displacement experiment. Finally, a novel protein fluorescent labeling technique was developed for the

identification of selective displacers, opening up the possibility of quickly screening a large amount of selective displacers in future studies.