An Investigation into the Effects of Protein Surface Modifications on Protein Binding Affinity in Ion Exchange and Multimodal Chromatography

by

Wai Keen Chung

An Abstract of a Thesis 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. Pete Tessier, Member

Prof. George Makhatadze, Member

Prof. Blanca Barquera, Member

Prof. Scott McCallum, Member

Professor K.V Lakshmi, Member

Rensselaer Polytechnic Institute
Troy, New York

July 2009
(For Graduation August 2009)

ABSTRACT

The development of efficient bioseparation processes for the production of high-purity biopharmaceuticals is one of the most pressing challenges facing the pharmaceutical and biotechnology industries today. This has led to the development of multimodal chromatographic systems which enable a combination of interactions (e.g. electrostatics, hydrophobicity, hydrogen bonding, etc) with a protein surface, giving rise to unique selectivities that are not seen on conventional chromatographic resins. While these new materials offer potential for bioseparations, there is a lack of fundamental understanding of the nature of binding of these ligands to protein surfaces. Homologous proteins which possess similar structures but varying surface properties provide a well defined library of molecules to examine multimodal chromatographic interactions. Different chemical modifiers were employed to generate lysozyme and horse cytochrome c charge ladders (homologous protein variants) to study the effects of protein binding in ion exchange chromatographic systems. In the study using lysozyme charge ladders, capillary zone electrophoresis (CZE) and mass spectrometry (MS) analysis of the cation exchange column eluents showed some protein variants eluting in an order contrary to conventional thinking while variants with different amounts of surface charge were also observed to be co-eluting in the same fractions. Enzyme digest-MS was carried out to determine the exact sites of modification on the protein surface and electrostatic potential maps of variants and native protein revealed important regions on the protein surface that played a significant role in determining protein retention on the ion exchanger. The retention behavior of horse cytochrome c variants on a cation exchanger was examined using a similar approach and important binding regions on the surface of the protein were defined. Homologous libraries of cold shock protein B (CspB) and ubiquitin mutants with a range of protein surface modifications were used to study differences in protein retention behavior on ion exchange as well as multimodal chromatographic systems. Examination of the elution trend of the CspB library on the ion exchange surface in concert with electrostatic potential mapping showed changes in protein retention to be dependent upon charge density and distribution as well as its locality and neighboring charged residues. Both CspB and ubiquitin libraries showed

stronger retention on the multi-modal chromatographic surface as compared to the ion exchanger, with significant differences in elution order. One hypothesis is that synergistic effects of multimodal interactions are the cause of increased protein retention on multimodal resins and that the ligand interacts with regions on the protein surface that complement each mode of interaction. In contrast, the ion exchange ligand will interact with charged regions on the protein surface. To test this hypothesis, HSQC NMR titration studies were performed using $^{13}C/^{15}N$ isotopically labeled ubiquitin and representative multimodal chromatographic and ion exchange ligands. Chemical shift mapping and determination of dissociation constants was carried out to determine the sites of interaction for the multimodal ligand and the ion exchange ligand on the protein surface. A major multimodal ligand interaction site was found on the protein surface in addition to a few weak interaction sites on other regions of the protein. In contrast, the interaction sites for the ion exchange ligand were more distributed and had binding affinities that were of an order of magnitude weaker than the multimodal ligand interaction sites. Identification of the ligand binding conformation as well as key chemical features within each ligand at its strongest interaction site was examined using a coarse-grained ligand docking program that was guided by the NMR experimental data. The multimodal ligand was shown to interact with the protein surface through various chemical moieties on the surface of the ligand and adopted two distinct high affinity binding conformations where as the ion exchange ligand primarily interacted with the protein surface through charge re-enforced hydrogen bonds. The insights gained from these studies provide deeper fundamental understanding into the nature of selectivity in multi-modal chromatographic systems. This will enable the design of next generation multi-modal ligands and facilitate methods development for protein purification with these novel systems.