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Defining the property space for chromatographic ligands from a homologous series of mixed-mode ligands

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ABSTRACT

A homologous ligand library based on the commercially-available Nuvia cPrime ligand was generated to systematically explore various features of a multimodal cation-exchange ligand and to identify structural variants that had significantly altered chromatographic selectivity. Substitution of the polar amide bond with more hydrophobic chemistries was found to enhance retention while remaining hydrophobically-selective for aromatic residues. In contrast, increasing the solvent exposure of the aromatic ring was observed to strengthen the ligand affinity for both types of hydrophobic residues. An optimal linker length between the charged and hydrophobic moieties was also observed to enhance retention, balancing the steric accessibility of the hydrophobic moiety with its ability to interact independently of the charged group. The weak pKa of the carboxylate charge group was found to have a notable impact on protein retention on Nuvia cPrime at lower pH, increasing hydrophobic interactions with the protein. Substituting the charged group with a sulfonic acid allowed this strong MM ligand to retain its electrostatic-dominant character in this lower pH range. pH gradient experiments were also carried out to further elucidate this pH dependent behavior. A single QSAR model was generated using this accumulated experimental data to predict protein retention across a range of multimodal and ion exchange systems. This model could correctly predict the retention of proteins on resins that were not included in the original model and could prove quite powerful as an in silico approach toward designing more effective and differentiated multimodal ligands.

1. Introduction

Multimodal chromatographic systems have developed in a variety of forms including mixed-mode, hydrophobic charge induction, mixed ligands and mixed bed chromatographic systems, with many permutations of ligand structures within each category [1–6]. The modes of interaction in these systems are typically either a combination of electrostatic and hydrophobic interactions or a mixture of positive and negative charges which can present unique advantages in selectivity over traditional single mode chromatographic separations [2–4,7]. Mixed-mode chromatography and hydrophobic charge induction chromatography are the predominant methods utilized in preparative scale multimodal separations, largely due to their superior resolution of impurities or the ability to capture proteins directly from high ionic strength cell culture fluid [8–13]. In these forms of multimodal chromatography, the orthogonal modes of interaction are combined into a single molecular entity, improving the homogenous distribution of both interaction moieties across the surface of the chromatographic support.

There is a growing set of publications in the literature that investigate the chemical and structural diversity of multimodal ligands and have begun to identify structural characteristics that lead to significant functional diversity [14]. In the work of Johansson et al., a comprehensive set of mixed-mode and mixed-ligand media was synthesized to create cation-exchange and anion-exchange libraries and the results indicated that ligands containing aromatic moieties demonstrated increased salt-tolerant adsorption as compared to ligands with aliphatic chain groups [4,15,16]. Mountford

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et al. [17] created a series of heterocyclic aromatic rings systems with a variety of substituents and geometric arrangements and observed that the more polar ligands tended to be more selective when capturing a target antibody and resolving it from cell culture fluid contaminants. Molecular dynamics simulations with MEP HyperCel, a pyridine-based ligand, showed that this ring forms both hydrophobic and hydrogen bonding interactions that help it form a tight interaction with the target hydrophobic pocket on the Fc-region of an antibody [18]. This ligand also incorporated a thioether group and was developed as part of a class of thiophilic ligands that utilize hydrophobic π-donor/acceptor interactions to form strong interactions with aromatic groups and were observed to specifically adsorb immunoglobulins from a background of host cell impurities [1,19–21]. In the first paper in this series [22], it was observed that spatial organization of hydrophobic and charged moieties on two multimodal cation-exchange ligands (Capto MMC and Nuvia cPrime) proved to have a substantial effect on the retention behavior of certain proteins with clusters of surface-exposed aliphatic residues while having similar affinities to charged and aromatic moieties.

However, many more variables in multimodal ligand design have yet to be characterized, three of which are addressed in the current study. These variables include the role of geometric constraints (the distance between two functional groups and the relative steric accessibility of these functional groups), the effect of charge density and ligand pKa, and the presence of a polar substituent near the hydrophobic moiety. In the current work, these variables are characterized using a homologous series of nine prototype ligands that are based on a commercial multimodal resin template (Nuvia cPrime) so that alternate sources of variation (base matrix chemistry, immobilization chemistry and ligand density) are greatly reduced and any differences can be associated with changes in the chemical and structural properties of these ligands. In addition, these ligands are screened across a diverse set of protein chemistries and structures which can then be used to identify class-specific differences in protein adsorption that are related to a particular change in ligand chemistry. Finally, a single QSAR model is generated using this accumulated experimental data to predict protein retention across a range of multimodal and ion exchange systems.

2. Materials and methods

2.1. Materials

Glacial acetic acid and guanidine hydrochloride were purchased from Thermo Fisher Scientific (Pittsburgh, PA). Sodium chloride, sodium acetate, sodium phosphate monobasic, sodium phosphate dibasic, sodium hydroxide, hydrochloric acid, L-arginine HCl, urea, ovalbumin (chicken egg white albumin), α-lactalbumin (bovine), albumin (bovine, human), conalbumin (chicken egg white), β-lactoglobulin A (bovine milk), β-lactoglobulin B (bovine milk), trypsin (bovine and porcine), α-chymotrypsin (bovine pancreas), α-chymotrypsinogen A (bovine pancreas), ribonuclease A (bovine pancreas), ribonuclease B (bovine pancreas), cytochrome C (horse heart), aprotinin (bovine lung), lysozyme (chicken egg white), papain (papaya latex), and avidin (egg white) were purchased from Sigma–Aldrich (St. Louis, MO). Recombinant human ubiquitin was purchased from Boston Biochem, Inc. (Cambridge, MA). Capto MMC, CM Sepharose Fast Flow and SP Sepharose Fast Flow chromatography media were purchased from GE Healthcare (Uppsala, Sweden). MX-Trp-650 M chromatographic media was a gift from Tosoh Biosciences LLC (King of Prussia, PA). Nuvia cPrime and the various prototype chromatography media were provided by our collaborator, Bio-Rad Laboratories (Hercules, CA). 5 mm × 50 mm glass columns and adapters were purchased from Pharmacia Biotech (Uppsala, Sweden).

2.2. Column packing procedure

Chromatographic resin was first equilibrated in deionized water and then was resuspended in a 50% (v/v) slurry in deionized water. 2.2 mL of slurry was poured into a 5 mm (ID) × 50 mm column and flow-packed in deionized water at 0.5 mL/min for 30 min. The flow adapter was adjusted onto the surface of the resin bed and flow was adjusted to 1 mL/min and packed for another 30 min. The adapter was again adjusted onto the bed surface at the final bed volume of ~1 mL.

2.3. Resin titration experiments

Chromatographic resin was first equilibrated in deionized water and then rinsed with an equal volume of 0.1 M HCl. The resin was then resuspended in an equal volume of 0.1 M HCl and equilibrated for 2 h with mild agitation to maintain the suspension of resin particles. Afterwards, the solution was allowed to settle and the supernatant was removed. An equal volume of 0.1 M HCl was added to the settled resin and the solution was resuspended. This solution was then titrated with 0.1 M NaOH. The solution was thoroughly mixed after each addition of base and the solution pH was recorded after a delay of 5 min.

2.4. Protein library screening experiments

Linear gradient experiments were performed on an Äkta Explorer 100 (Amersham Biosciences, Uppsala, Sweden). Running buffers for all experiments were prepared from a 25× concentrate (500 mM acetate, pH 5 or 500 mM phosphate, pH 6) and diluted to the desired concentration without further pH adjustment. Buffers containing co-solutes (urea, guanidine-HCl and L-arginine HCl) were pH adjusted as needed using 2 M NaOH or 2 M HCl stock solutions.

1 mL columns were equilibrated at 1 column volume (CV)/min with 5 CV of 1% Buffer B (Buffer A + 1.5 M NaCl) in Buffer A prior to the start of each experiment. Proteins were dissolved in the equilibration buffer (1% Buffer B) to 3 mg/mL and pipetted into 96-well UV transparent well plates. 50 μL of protein solution was aspirated by the A-905 autosampler (Amersham Biosciences, Uppsala, Sweden) and injected into the column. A linear salt gradient from 1 to 100% Buffer B was generated over 45 CV and held at 100% B for 8 mL (to account for the dead volume of the chromatography system). The column was then re-equilibrated with 7 CV prior to the next injection. Absorbance at the column effluent was measured at 280 nm and 215 nm using a 10 mm UV flow cell. Retention times were determined by calculating the center-of-mass for each peak. The conductivity in mS/cm was determined for that retention time and the conductivity was used to determine the elution salt concentration value.

2.5. pH gradient experiments

Linear gradient experiments were performed on an Äkta Explorer 100 (Amersham Biosciences, Uppsala, Sweden). Running buffers for all experiments were prepared from a 20× concentrate (400 mM each of citrate, phosphate, tris base and glycine, titrated to either pH 4.0 or pH 11.0) and diluted to the desired concentration without further pH adjustment.

1 mL columns were equilibrated at 1 column volume (CV)/min with 5 CV of Buffer A (pH 4.0 buffer) prior to the start of each experiment. Proteins were dissolved in the equilibration buffer to 3 mg/mL and deposited into 96-well UV transparent well plates.