Journal of Chromatography A, 1569 (2018) 101–109

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Investigating the impact of aromatic ring substitutions on selectivity for a multimodal anion exchange prototype library

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ARTICLE INFO

Article history:
Received 24 May 2018
Received in revised form 8 July 2018
Accepted 15 July 2018
Available online 17 July 2018

Keywords:
Multimodal chromatography
Ligand design
Hydrophobicity
Quantitative structure activity relationship (QSAR) model

ABSTRACT

The increasing prevalence of low pI non-mAb therapeutics as well as current challenges in mAb-aggregate separations and low recoveries motivate further development in the multimodal anion exchange (MM AEX) space. In this work, linear salt gradient experiments at pH 7 were used to evaluate the retention of model proteins (with pI from 3.4 to 6.8) in 17 novel MM AEX prototype systems. The ligands were organized into three series. Series 1 extended previous work in multimodal ligand design and included a hydroxyl variant and linker length variants. Series 2 and 3 investigated the nature of hydrophobicity in MM AEX systems by adding hydrophobic (series 2) or fluorine (series 3) substituents to a solvent exposed phenyl ring. Compared to the commercial resin Capto Adhere, the series 1 and 3 ligands exhibited weaker binding, while some of the series 2 aliphatic prototypes showed dramatically increased retention and unique selectivities. Within series 1, the model proteins eluted earlier in the gradient as the charge-hydrophobic group distance on the ligand was increased from 4.9 Å to 8.5 Å. For the aliphatic variants in series 2, proteins that eluted early in the salt gradient were not affected by the increase in ligand hydrophobicity, while the later eluting proteins bound stronger as the length of the aliphatic substituent increased. The series 3 variants indicated that phenyl ring fluorination created subtle changes in protein elution in these MM AEX systems. Retention data from the three series was used to generate a partial least squares QSAR model based on both protein and ligand descriptors which accurately predicted protein retention with a training R^2 of 0.81 and a test R^2 of 0.76. The retention characteristics of some prototypes such as the earlier elution and unique selectivities compared to Capto Adhere suggest that they could potentially provide unique selectivities and increased recovery for the downstream processing of both mAb and non-mAb biotherapeutics.

1. Introduction

In recent years, the use of multimodal resins in downstream processing has increased due to their unique selectivities, ability to clear process impurities, and higher binding capacities at elevated salt concentrations [1,2]. The increased understanding and commercial application has also motivated the development of novel multimodal ligands with different selectivities and improved performance (binding capacity, recovery, etc.). A comprehensive review of multimodal ligand structures and design principles was published in 2009 [3]. Since then, multimodal ligand development has continued to progress at a rapid pace, utilizing both *in silico* and experimental design techniques [4].

Several groups have focused on the development of hydrophobic charge induction (HCIC) materials. Mountford et al. investigated structural variants of pyridine-based and other N-heterocyclic resins and evaluated these prototypes as hydrophobic charge induction (HCIC) chromatographic capture steps for antibodies [5,6]. In a series of recent papers, an array of HCIC ligands were synthesized and the effects of ligand structure, density, and pKa on the resin binding capacities and salt tolerance were examined [7–9].

The design of multimodal cation exchange (MM CEX) resins with new selectivities also has been an active area of multimodal ligand research. In a recent paper from our group, the solvent exposure and

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https://doi.org/10.1016/j.chroma.2018.07.049
0021-9673/

spatial orientation of the charge and aromatic moieties was found to dramatically affect the retention behavior of model proteins in two MM CEX systems [10]. In related work, ligand structure was investigated by systematically varying the charge and hydrophobic group linker length, cationic charge group and ligand pKa, and the proximity of a polar group to the aromatic ring [11]. In addition to small molecule ligands, resins with polymeric MM CEX ligands have also been investigated [12,13]. Although these resins exhibit unique properties, the interaction mechanism is more complex and predicting column behavior is often difficult for either ion exchange [14] or multimodal systems.

The increasing prevalence of low pI non-mAb therapeutics [15–17] as well as the need for more efficient mAb-aggregate flow through operations [18] motivates the development of a more diverse multimodal anion exchange ligand library. Early work in MM AEX ligand development explored both aliphatic and aromatic hydrophobic moieties and identified low recoveries as a potential challenge for these resins. Johansson et al. developed an extensive library of MM AEX prototypes and evaluated this library based on binding capacity and recovery [19]. In related work, Yang et al. evaluated recovery for a series of aromatic MM AEX ligands and found that high concentrations of $MgCl_2$ were required to obtain good recoveries of the model protein lectin [20]. The selectivity of MM AEX ligands has also been studied. Hou et al. investigated the Capto Adhere MM AEX system with a series of model proteins and developed a QSAR model to predict protein retention in the Capto Adhere resin system [21]. Pezzini et al. recently characterized the Pall PPA and HEA HyperCel resins, studying protein binding under a wide range of salt, mobile phase modifier, and pH conditions [22] and also evaluated these ligands as capture steps for antibody products [23]. O'Connor and coworkers compared the performance of several MM AEX resins for an aggregate removal step incorporated in a mAb platform and used mobile phase modifiers to provide insight into the separation mechanism [24].

The current work builds on the previous investigations of both multimodal cation and anion exchangers by using a model protein library with a range of pI and surface hydrophobicity to evaluate selectivity for three series of novel MM AEX prototype ligands. The first series included ligands with varied linker length and the addition of a hydroxyl moiety. Prototype ligands in the remaining series (with the exception of a secondary amine charge variant) explored the effect of adding substituents to a solvent exposed phenyl ring. Series 2 included aliphatic and aromatic variants and investigated the nature of hydrophobicity in MM AEX systems. Series 3 included fluorinated variants and explored the effect of phenyl ring polarizability. Model protein retention in the various prototype systems then was used to generate a partial least squares QSAR model to predict protein retention. The selected descriptors were employed to provide insight into the important ligand and protein characteristics for these prototype systems. This work advances the understanding of hydrophobicity in multimodal ligand design by evaluating the effect of aromatic ring substituents and lays the foundation for the future application of these resins to challenging industrial bioseparations problems.

2. Materials and methods

2.1. Materials

Capto Adhere multimodal anion exchange resin was purchased from GE Healthcare (Uppsala, Sweden). Sodium phosphate, sodium chloride, beta lactoglobulin A (from bovine milk), beta lactoglobulin B (from bovine milk), bovine serum albumin (BSA), cellulase (from *Trichoderma reesei* ATCC 29621), conalbumin (from chicken egg white), human serum albumin (HSA), lectin (from *Phaseolus vulgaris*, red kidney bean), ovalbumin (turkey), transferrin (human), and trypsin inhibitor (Type III-O: chicken egg white) were purchased from Sigma Aldrich (St. Louis, Mo.). Multimodal anion exchange prototypes were synthesized by Bio-Rad Laboratories (Hercules, Ca.).

2.2. Chromatography experiments

MM AEX columns were packed in 5 x 50 mm glass columns for a total column volume of 0.8–1.1 mL and an asymmetry of 0.85–1.15 as determined by first moment analysis of an acetone pulse. Analytical chromatography experiments were performed using an Äkta Explorer 100 controlled by Unicorn 5.31 software. Linear salt gradients from 0 to 100% Buffer B were generated over 40 column volumes (CV) at a flow rate of 1 CV/min. Equilibration buffer (Buffer A) was 10 mM phosphate, pH 7 and Buffer B was 10 mM phosphate, pH 7 with 1 M NaCl. Phosphate buffers were prepared by diluting a 100 mM phosphate stock solution, filtered using 0.2 μm filters (Whatman; Florham Park, NJ), and pH adjusted using 2 N HCl or NaOH. Model protein stock solutions were prepared by dissolving lyophilized protein in equilibration buffer at a concentration of 3–6 mg/mL and used within two weeks of preparation. For each experiment, 100–200 μL of protein sample was injected. Elution salt concentrations were calculated using conductivities corresponding to the peak first moment. Results are presented as the average of duplicate runs. Error bars represent the standard deviation of duplicate runs.

2.3. Descriptor calculations

Ligand descriptors were calculated in the Molecular Operating Environment (MOE) software package from Chemical Computing Group (Montreal, Canada). Global protein descriptors also were calculated in MOE. Custom designed residue cluster descriptors, individual property map, and overlapping cluster protein descriptors were calculated in house according to the procedure previously described [10]. A total of 62 protein descriptors and 20 ligand descriptors were calculated for each protein or ligand. Table 1 lists the model protein library employed in this work and the PDB structures used for descriptor calculations.

2.4. QSAR model development

QSAR modeling was conducted using the partial least squares (PLS) algorithm implemented in the Scikit-Learn Python package [25]. The total data set comprised the retention of 10 proteins on the 17 MM AEX prototype resins and Capto Adhere. Only proteins that eluted during the gradient were used during model development. Protein/ligand pairs for which the protein did not elute were omitted from the training or test sets. The training set contained retention data for the complete model protein library on 15 resins. The test set was comprised of protein retention on two resins (86 1 and 91 2) that were excluded from the training set.

The PLS model projects both the x-data (here, the protein property descriptors) and the y-data (here, the elution on a MM AEX resin under the gradient conditions defined above) into a new dimension. The Variable Influence on Projection (VIP) score provides a weighted measure of how each descriptor contributes to both models and a higher value indicates that a descriptor is more important. In this work, the VIP score was used as a method of feature selection. A PLS model first was developed using the complete set of 82 protein and ligand descriptors that were calculated. The VIP score was used to reduce the number of descriptors in successive PLS models, with a threshold score of 1 for protein descriptors and 0.9 for ligand descriptors. A lower threshold for ligand descriptors was used because these descriptors were observed to be less