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Protein adsorption in polyelectrolyte brush type cation-exchangers

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ABSTRACT

Ion exchange chromatography materials functionalized with polyelectrolyte brushes (PEB) are becoming an integral part of many protein purification steps. Adsorption onto these materials is different than that onto traditional materials, due to the 3D partitioning of proteins into the polyelectrolyte brushes. Despite this mechanistic difference, many works have described the chromatographic behavior of proteins on polyelectrolyte brush type ion exchangers with much of the same methods as used for traditional materials. In this work, unconventional chromatographic behavior on polyelectrolyte brush type materials is observed for several proteins: the peaks shapes reveal first anti-Langmuirian and then Langmuirian types of interactions, with increasing injection volumes. An experimental and model based description of these materials is carried out in order to explain this behavior. The reason for this behavior is shown to be the 3D partitioning of proteins into the polyelectrolyte brushes: proteins that fully and readily utilize the 3D structure of the PEB phase during adsorption show this behavior, whereas those that do not show traditional ion exchange behavior.

1. Introduction

Ion exchangers (IEX) have been used for the purification of many analytes, including therapeutic proteins, since the beginnings of preparative scale chromatography. Traditionally, these ion exchangers consist of spherical, often porous, particles packed into a column and functionalized with IEX ligands. The base matrix composition varies broadly from silica gels to cross-linked agarose to organic polymers such as polyvinyl ether and methacrylate polymers [1]. On the other hand, the IEX ligands can be sorted into two categories: strong (i.e. sulfonates) and weak ions (i.e. carboxylic acids) [2].

Two characteristics of performant IEX materials are a high analyte-surface interaction area (high capacity), and large pore sizes allowing larger analytes such as proteins to enter the particles unhindered (low mass transfer limitations) [1–4]. These are unfortunately often in contradiction as large pores lead to small surface areas. In order to overcome this limitation, Müller [5], based on previous works by Vanecek and Regnier [6] and Alpert [7], developed a new type of IEX phase with "tentacle-like" arrangement of the ionic surface groups: instead of the standard type ligands which are fixed onto the surface via spacer arms, Müller bound polyelectrolytes onto the surface. With each ligand containing more than one ionic group, adsorption capacity no longer solely depended on the interaction surface area, but rather on the interaction volume created by the polyelectrolyte brushes (PEB). This was described by Lenhoff [8] as 3-D partitioning of the analyte into the PEBs as opposed to the traditional 2-D surface adsorption. Increased mass transfer rates were also observed and can be explained by larger pore sizes (pore diffusion depends on the ratio between molecule and pore size [9]). Because of these advantages, PEB type phases have become very popular in the last few years, with several IEX phase producers having brought this type of material to market. De Neuville et al. pushed this concept further by developing a perfusive material with micron size pores and PEB ligands that displayed excellent mass transfer characteristics while retaining good capacities [10].

Despite this increase in popularity, very little work detailing the chromatographic behavior of proteins on PEB materials has been done. It is generally assumed that adsorption onto PEB type surfaces can be described in the same manner as onto non-PEB type surfaces [8,11–16]. In this work, cases where atypical chromatograms are observed on cation-exchange PEB type materials will be shown. With small injection volumes, an anti-Langmuirian peak shape is first observed. A shift then occurs with increasing injection volumes, and Langmuirian peak shapes are observed. This behavior will be shown on all PEB type phases tested and in different conditions with gradient, isocratic and flow-through experiments. Several possible reasons for this type of behavior will be explored. This behavior is then explained by multi-layer adsorption and an adsorption isotherm is developed. Using this isotherm

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Table 1
Information on the PEB type resins used in this work.

Name	Manufacturer	Base Matrix	Avg. Particle Size	Avg. Pore Size
SP Sepharose XL	GE Healthcare	Cross-linked agarose	90 μm	N/A
Eshmuno CPX	Merck Millipore	Polyvinyl ether	50 μm	80 nm
Fractogel EMD SO_3^- (M)	Merck Millipore	Polymethacrylate	40–90 μm	80 nm
Fractogel EMD COO^- (M)	Merck Millipore	Polymethacrylate	40–90 μm	80 nm
Toyopearl GigaCap S-650-M	Tosoh	Polymethacrylate	75 μm	100 nm

along with a mass balance and qualitative information, the reason behind the presence or absence of this behavior is mechanistically explained. Finally, the impact of this behavior on protein separation is shown through the separation of a mixture of model proteins.

2. Materials and apparatus

2.1. Stationary phases

Five different PEB-type phases where used in this work. These are shown in Table 1. All information is easily found on the manufacturers website [17–19].

In addition, the Toyopearl SP-650M (Tosoh Bioscience, polymethacrylate, 65 μm, 100 nm) was also used for comparison, as it is similar to the GigaCa, but. without PEB ligands. All are strong cation exchangers, with sulfonates as the functional group, except for the Fractogel EMD COO^- (M), which is a weak cation exchanger, with carboxylic acid as the functional group.

All the columns where purchased prepacked from Atoll (Weingarten, Germany) and were 1 mL in volume (0.5 × 5 cm dxl). Many of the experiments in this work were also carried out on larger columns with the same or similar results (results not shown in this work).

2.2. Proteins

Lysozyme from chicken egg white (>98%) (LYSO) was purchased from Fluka Analytical (Buchs, Switzerland). Chymotrypsinogen A from bovine pancreas (CHY A) was purchased from AppliChem (Darmstadt, Germany). Cytochrome C from horse heart muscle (>90%) (CYT C) was purchased from Acros Organics (Geel, Belgium). Goserelin (crude, ~50%) (GOS) was kindly donated by Corden Pharma Switzerland (Liestal, Switzerland). The two monoclonal antibodies (mAb1 and mAb2) used were produced and purified in house (pI > 8.0 for both).

LYSO, CHY A, CYT C and GOS were available as freeze dried powders and were dissolved into the loading buffers at the different pH values (see Section 2.3 for mobile phase compositions). mAb1 and mAb2 were available as solutions and were buffer exchanged into the loading buffers at the different pH values using Amicon Ultra-15 centrifugal filters with a 50 kDa cutoff (Merck Millipore, Billerica, MA, USA). Unless otherwise stated, the protein concentrations were always 5 g/L.

2.3. Mobile phases

Mobile phases buffered at pH 5, 7 and 8 were prepared with acetate, phosphate and tris salts respectively. The buffering concentration was always 25 mM. At each pH, a loading (no added NaCl) and a strong (1M NaCl at pH 5 and 7, 0.5 M at pH 8) buffer were prepared. Acetic acid was purchased from Sigma Aldrich (Buchs, Switzerland). NaCl was purchased from Fisher Chemical (Pittsburgh, PA, USA). Sodium acetate was purchased from Merck KGaA (Darmstad, Germany). Sodium phosphate mono and di-basic were purchased from Fluka (Buchs, Switzerland). Tris base was purchased from Biosolve BV (Valkenswaard, Netherlands). Tris HCl was purchased from Acros (Geel, Belgium). All chemicals were used without further purification. De-ionized water was further purified with a simpak2 unit (Synergy Millipore, MA, USA). Unless otherwise stated, the flow rate was always 0.5 mL/min.

2.4. HPLC

All experiments were carried out on an Agilent (Santa Clara, CA, USA) 1100 series HPLC, equipped with an auto-sampler, a column thermostat, a variable wavelength detector, an online degasser and a quaternary gradient pump. A Gilson FC 203 B fraction collector (Middleton, WI, USA) was connected (if needed) at the HPLC outlet to collect fractions during experimental runs.

2.5. Batch uptake experiments

The equilibrium binding capacity of LYSO was measured on several resins using batch uptake experiments. This was done at pH 7.0 at different ionic strengths. 1.8 mL of LYSO solution (at different concentrations) was added to 0.1 mL of pre-equilibrated resin (i.e. pH 7.0 and same ionic strength). The mixture was left under heavy mixing for 24 h. The liquid phase protein concentration was determined by UV absorbance at 280 nm. The amount of adsorbed protein was then calculated from the mass balance.

3. Experimental results

3.1. Anti-Langmuir to Langmuir behavior

LYSO and GOS, at pH 7 and CHY A at pH 5 in their respective loading buffers were injected onto pre-equilibrated (loading buffer) PEB-type chromatographic materials. Injections from 0.1 to 25 mL were done, resulting in load values of 0.5–125 g of analyte per L of bed volume. These were then eluted with a 30 min gradient. The results are shown in Fig. 1. Only the results on the Eshmuno CPX material are shown. All other PEB type materials exhibit similar behavior and can be found in the Appendix.

It is clear from Fig. 1 that the adsorption of these three analytes onto PEB type materials is not typical. In fact, it is expected that protein adsorption onto IEX materials should follow a Langmuirian behavior, as is described by the steric-mass action model [20]. Instead, a more complex transient behavior is observed: at smaller loads, the analytes behave in an anti-Langmuir type manner, and, as the injections volumes increase, the behavior shifts to a Langmuirian type. This behavior will be referred to as the AL-L behavior in the remainder of this work. The point at which the behavior shifts from anti-Langmuirian to Langmuirian will be referred to as the critical point. For GOS, the feed solution was not pure, which led to low capacities and breakthrough at higher injection sizes. As such, in this case, only the initial anti-Langmuirian type behavior is observed (pre- critical point). As this behavior is observed for all three analytes, only the results for LYSO are shown in subsequent sections.

Based on this behavior it would then be expected that the adsorption equilibrium isotherm of proteins onto this type of material would contain an inflection point, with the adsorption isotherm