Journal of Chromatography A, 1253 (2012) 83–93

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Counterion effects on protein adsorption equilibrium and kinetics in polymer-grafted cation exchangers

Ernie X. Perez Almodovar[1], Bianca Glatz, Giorgio Carta*

Department of Chemical Engineering, University of Virginia, Charlottesville, VA 22901, USA

ARTICLE INFO

Article history:
Received 29 April 2012
Received in revised form 28 June 2012
Accepted 29 June 2012
Available online 11 July 2012

Keywords:
Proteins
Ion exchange
Counterions
Equilibrium
Kinetics
Grafted media

ABSTRACT

Protein binding equilibrium and mass transfer kinetics are studied for cation exchangers containing charged polymer grafts as well as for a macroporous matrix in pH 5 acetate buffers using sodium, tetra-n-butylammonium (TBAH), arginine, and calcium as counterions and a monoclonal antibody (mAb) as a model protein. Dynamic light scattering shows that there is no significant effect of the counterion type on the mAb aqueous diffusivity. The counterion type also does not affect substantially the structure of the polymer grafts, nor does it affect the stoichiometry of the protein ion exchange process. While no counterion effects are also observed on the protein mass transfer kinetics for the macroporous matrix, very large effects are seen for the polymer grafted matrices with protein adsorption rates increasing dramatically in the order $Ca^{++} > Arg^{+} > Na^{+} > TBAH^{+}$. This order is the same order in which the relative protein binding strength decreases. Accordingly, the counterions leading to weaker protein binding also lead to faster protein diffusion. Although the quantitative aspects are different, the same trends hold for different proteins (lysozyme and lactoferrin) and for an agarose-based matrix also containing grafted polymers (Capto™ S). The underlying mechanism is qualitatively consistent with protein transport occurring through a hopping process driven by the adsorbed protein concentration within the apparently flexible network structure formed by the grafted polymers. From a practical viewpoint, the results show that improved protein adsorption kinetics in polymer-grafted cation exchanger and, hence, improved performance, can be obtained by selecting particular counterions.

1. Introduction

Ion exchangers are used extensively in the capture and purification of proteins [1–3]. At the process scale, equilibrium binding capacity and adsorption rates are generally critical since they, together, determine dynamic binding capacity and productivity [3]. As recently reviewed by Lenhoff [4], polymer-functionalized ion exchangers, where charged polymers are introduced either by grafting to a support matrix or by in situ polymerization, exhibit, in some instances, both high protein binding capacity and faster protein adsorption rates compared to more traditional macroporous matrices. The exact mechanism responsible for the improved performance is not known [4]. However, it has been suggested that the grafted charged polymers act as surface extenders forming a continuous three-dimensional network, akin to a hydrogel, which is stabilized by the support matrix and where protein binding and transport occur simultaneously [4–6]. In this case, as long as protein molecules retain diffusional mobility within the polymer phase, faster mass transfer occurs because of the large driving force associated with the high adsorbed protein concentration [7–10].

Although van der Waals, hydrogen bonding, and steric interactions are most likely contributing factors, the fundamental driving force for protein binding in ion exchangers is a stoichiometric exchange with counterions described by the stoichiometric displacement or mass action law model [11–14]. Sodium ion is most commonly used as the counterion in cation exchange chromatography, but the potential practical usefulness of other cations has been suggested. Arakawa et al. [15], for example, noted that higher valence cations could be used effectively to modulate elution. The same authors also suggested using arginine, which is cationic at pHs typically used for protein cation exchange, to help prevent aggregate formation. Annathur et al. [16] also suggested using arginine as an efficient eluent in the cation exchange chromatographic purification of a PEGylated peptide. In their study, compared to sodium chloride, arginine acetate in the load buffer actually reduced somewhat the peptide binding capacity. However, since arginine appeared to weaken the protein–resin interaction, earlier and sharper peaks were obtained upon elution. In turn, this effect decreased the PEGylated peptide hydrodynamic radius and thus improved mass transfer out of the particle pores. Addition of

* Corresponding author. Tel.: +1 4349246281; fax: +1 4349822658.
E-mail address: gc@virginia.edu (G. Carta).
[1] Current address: Amgen Manufacturing Limited, PO Box 4060, Juncos, PR 00777-4060, USA.

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.06.100

Symbols

A	parameter in mass action law model
C_i	liquid phase concentration of component i, mol/L or mg/mL
C_0	total equivalent concentration, equiv/L
CV	number of column volumes of mobile phase passed
D_0	diffusion coefficient, cm^2/s
k'_i	retention factor of component i
$K_{i,j}$	ion exchange equilibrium constant
L	column length, cm
q_i	concentration in adsorbed phase, mol/L or mg/mL
q_0	charge density, mol/L
$\hat{q}_i$	concentration averaged over the particle volume, mol/L or mg/mL
r_s	hydrodynamic radius, nm
v	mobile phase velocity, cm/s
z_i	charge of ion i
β	gradient slope, mol/L s
ε	extraparticle porosity
ε_p	porosity of backbone matrix
ϕ_g	volume fraction of adsorbent phase in the particle
γ	normalized gradient slope ($=\beta L/v$), mol/L
σ_P	hindrance parameter in SMA model

arginine and guanidine to mobile phases used in cation exchange chromatography has also been studied by Holstein et al. [17]. Significant effects of these species were seen on retention, but the behavior was highly protein-specific leading to the suggestion by these authors that these effects are caused by preferential interactions of arginine with specific regions on the protein surfaces and its effects on the surrounding hydration layer.

A seemingly important aspect that, to our knowledge, has not been explicitly investigated is the role of the counterion type on protein transport. This role is expected to be particularly important for polymer-functionalized ion exchangers, where protein transport occurs while simultaneously interacting with the charged polymer chains. Since protein binding equilibrium and transport are likely to be intimately coupled in these systems, it is reasonable to expect that the nature of the counterion will affect both. In this study we consider the effects of four different counterions on the binding behavior of a monoclonal antibody (mAb) in the commercial polymer-functionalized cation exchanger Nuvia™ S. The counterions studied are sodium, arginine, tetra-n-butylammonium ion, and calcium. These species are different in molecular size, aqueous diffusivity, and charge. Our measurements encompass inverse size exclusion chromatography to determine potential effects on the structure of the grafted polymer phase, frontal analysis counterion-exchange experiments to determine the relative affinity of each counterion for the stationary phase, linear gradient elution experiments to determine the protein effective charge, mAb adsorption isotherms to determine the effects on binding capacity, and mAb batch uptake rates to determine the impact of the counterion on intraparticle transport. The results are then extended to other matrices, with and without functionalizing polymers, and to two other model proteins, lysozyme and lactoferrin.

2. Experimental

2.1. Stationary phases

The cation exchangers used in this work are Nuvia™ S and UNOsphere™ S from Bio-Rad Laboratories (Hercules, CA, USA) and Capto™ S from GE Healthcare (Piscataway, NJ, USA). Nuvia™ S and UNOsphere™ S are based on a similar macroporous polymeric backbone. However, Nuvia™ S contains grafted, charged polymeric surface extenders. Capto™ S is based on an agarose backbone grafted with charged dextran polymers. All three materials contain SP-functional groups. Mean particle sizes are 85, 75, and 89 μm for Nuvia™ S, UNOsphere™ S, and Capto™ S, respectively. Other relevant properties of these materials are given in Refs. [18,19].

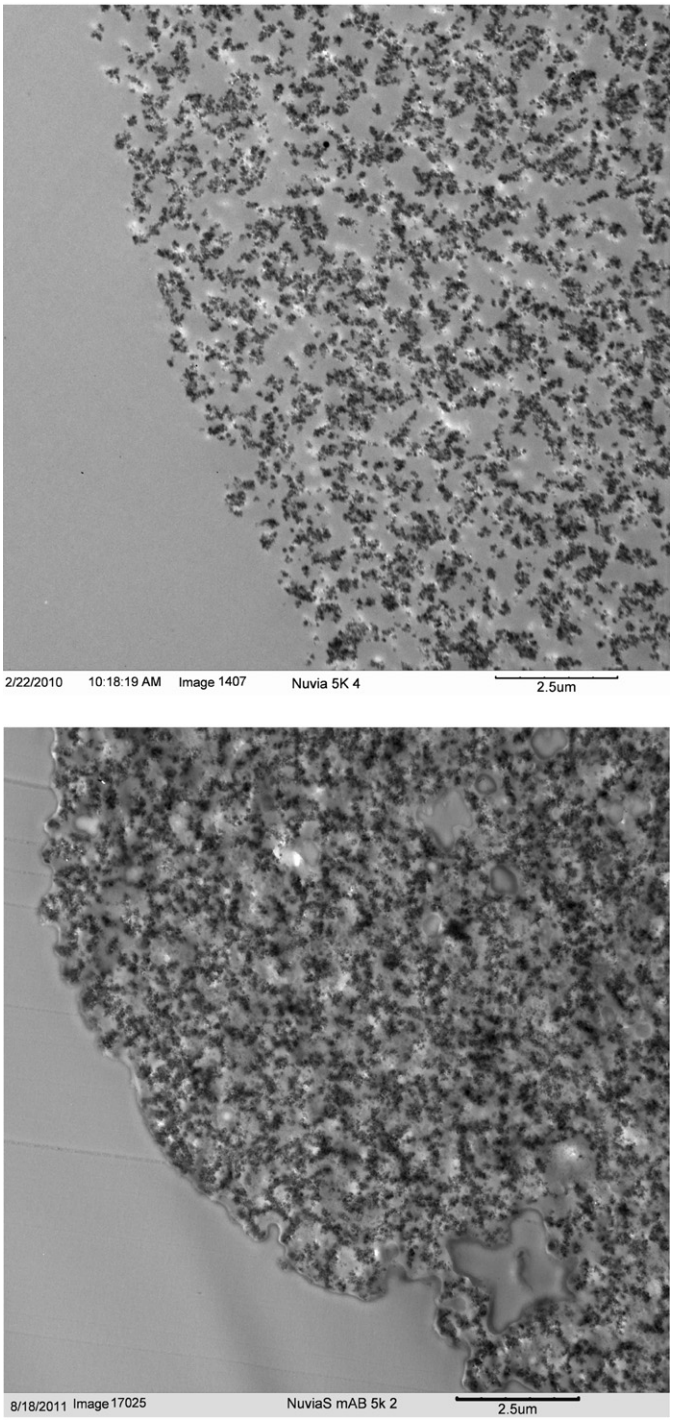

Fig. 1. TEM images of sections of clean (top) and mAb-saturated (bottom) Nuvia™ S particles. Images are taken near the particles external surface.

Fig. 1 shows transmission electron micrographs of 80 nm sections of Nuvia™ S obtained as described in detail by Perez-Almodovar et al. [19] by embedding the particles in LRWhite resin (obtained from London Resin Company, London, UK) and