Journal of Chromatography A, 1326 (2014) 29–38

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Adsorption of polyethylene-glycolated bovine serum albumin on macroporous and polymer-grafted anion exchangers

Mimi Zhu, Giorgio Carta*

Department of Chemical Engineering, University of Virginia, Charlottesville, VA 22904, USA

ARTICLE INFO

Article history:
Received 23 September 2013
Received in revised form
27 November 2013
Accepted 4 December 2013
Available online 22 December 2013

Keywords:
PEGylated proteins
Anion exchange
Polymer grafts
Diffusion

ABSTRACT

The chromatographic and adsorptive properties of BSA and BSA conjugated with 10 and 30 kDa PEG polymers are determined for a macroporous anion exchanger (UNOsphere™ Diol Q) and for a polymer-grafted material having the same backbone matrix (Nuvia Q™). Chromatographic retention, adsorption capacity, and adsorption kinetics are enhanced in the polymer-grafted resin for both BSA and 10 kDa PEG-BSA as a result of interactions with the grafted polymers. However, the difference between the two resins diminishes for 30 kDa PEG-BSA indicating that size exclusion effects strongly affect binding in the polymer-grafted material for this larger conjugate. Images of intraparticle concentration profiles obtained by confocal scanning laser microscopy show that the transport mechanisms of both BSA and PEGylated BSA are very different in the two resins. The protein binding kinetics are dominated by ordinary pore diffusion and are essentially independent of the direction of transport for UNOsphere Diol Q as a result of its large pore size. Thus, for this material, displacement of PEGylated BSA by BSA is clearly evident at the intraparticle scale. On the other hand, the protein binding kinetics in Nuvia Q are consistent with a solid diffusion mechanism driven by the adsorbed protein concentration. For this material, protein transport is very fast for one component or two-component co-adsorption of BSA and PEGylated BSA but slows down dramatically for sequential adsorption of these species as a result of heightened diffusional hindrance when the two components counterdiffuse within the resin.

1. Introduction

The conjugation of poly(ethylene glycol) chains, commonly referred to as PEGylation, is an established method to improve therapeutic proteins [1–3]. PEGylation can improve pharmacokinetics and pharmacodynamics by prolonging in-vivo half-life and reducing immunogenicity, and improve shelf life by increasing water solubility and reducing aggregation [4,5]. These beneficial effects result mainly from increased molecular size and surface protection, which occur when the neutral, chemically inert PEG polymers are conjugated with the protein. However, as noted by several authors [2,3,5,6], the same altered molecular properties that make PEGylation attractive from a therapeutic viewpoint, also introduce downstream processing complexities. Since PEGylation reactions are seldom completely quantitative, separation of PEGylated protein from native protein and unreacted PEG is often needed. Moreover, when multiple PEGylated species are formed as a result of random or residue-specific PEGylation, the separation of species with different degree of PEGylation as well as the separation of positional isomers may be needed [6,7]. While multiply PEGylated forms can be reduced by implementing site-directed PEGylation, either through site-directed mutagenesis [8,9] or via the incorporation of non-natural amino acids [10,11], complete separation still remains a challenge.

The separation of PEGylated and native protein species can be based on differences in size, charge, or a combination of both. Size exclusion chromatography (SEC) is a direct way of separating PEGylated protein from native protein, but its low productivity makes it undesirable for large-scale manufacture. Thus, SEC is primarily used as an analytical tool while separations based on electrostatic interactions by ion exchange chromatography (IEC), are usually preferred for preparative applications [5,6,12], especially when PEGylation involves reaction with the protein amine groups, which, in turn, changes the protein net charge. Even when PEGylation occurs by reaction with free sulphydryl groups, which are not charged at the pH values typically used in protein chromatography, specific interactions with the resin surface are often quite different for native and PEGylated molecules as a result of the partial shielding of charged residues caused by the PEG chains. It has been shown that retention of PEGylated species on IEC resin is weaker than that of the corresponding native protein, that it decreases with PEG molecular mass, and that it decreases for multiply PEGylated

* Corresponding author. Tel.: +1 4349246281; fax: +1 4349822658.
E-mail address: gc@virginia.edu (G. Carta).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.12.007

Nomenclature

C	protein concentration in solution excluding mass of conjugated PEG (mg/mL)
$C^E_{Cl^-}$	chloride concentration at elution (mM)
$C^f_{Cl^-}$	final chloride concentration (mM)
$C^0_{Cl^-}$	initial chloride concentration (mM)
CV	number of column volumes
CV_G	duration of gradient in CV units
d_p	particle diameter (cm)
D_e	apparent effective pore diffusivity (cm^2/s)
D_0	free solution diffusivity (cm^2/s)
h	reduced HETP ($=H/d_p$)
k_f	boundary layer mass transfer coefficient (cm/s)
k'	protein retention factor
k'_{Cl^-}	retention factor of chloride ion
k'_∞	protein retention factor for non-binding conditions
K	parameter in Langmuir isotherm (mL/mg)
K_D	partition coefficient between particles and solution in SEC
q	bound protein concentration excluding mass of conjugated PEG (mg/mL)
q_m	parameter in Langmuir isotherm (mg/mL)
r_h	hydrodynamic radius (nm)
r_p	particle radius (cm)
$r_{pore,m}$	radius of small pores (nm)
$r_{pore,M}$	radius of large pores (nm)
v	mobile phase velocity (cm/s)
v'	reduced velocity ($=vd_p/D_0$)
V_C	column volume (mL)
V_R	retention volume (mL)
z	protein effective binding charge
ε	extraparticle column porosity
$\varepsilon_{p,m}$	intraparticle porosity associated with small pores
$\varepsilon_{p,M}$	intraparticle porosity associated with large pores
γ	normalized gradient slope ($=\varepsilon(C^f_{Cl^-}-C^0_{Cl^-})/CV_G$)

species on both anion and cation exchangers compared to mono-PEGylated forms [7,13]. The separation of positional isomers is also possible by IEC since the effective surface charge of different isomers can vary with the site of conjugation [6,7,13]. Maiser et al. [13], for example, described the separation of five positional isoforms of mono-PEGylated lysozyme by pH gradient elution chromatography with a 10 μm diameter cation exchanger. However, although the separation was possible, the selectivity was relatively low requiring very high plate numbers usually only obtained at the analytical scale.

PEGylation also plays a role in the binding capacity and energetics of adsorption on IEC resins. Blaschke et al. [14] recently reported the equilibrium binding capacity of bovine serum albumin (BSA) PEGylated with a 12 kDa PEG for several commercial anion exchangers. The capacity varied from resin to resin but was always lower for the PEGylated protein compared to the native form. The authors concluded that for strong anion exchangers, BSA binding is exothermic and enthalpy driven while the adsorption of PEG-BSA is either endothermic or exothermic dependent on the particular resin. In general, polymer grafted matrices gave higher capacities for both forms.

Finally, PEGylation and the accompanying large increase in molecular size are also likely to affect diffusional hindrance in the stationary phase and, hence, reduce resolution and dynamic binding capacity. Pabst et al. [5], using PEGylated BSA and a range of commercial anion exchange resins at a 1 min residence time, observed reductions in dynamic binding capacity (DBC) between 48% and 92% for 12 kDa PEG-BSA and between 74% and 99% for 30 kDa PEG-BSA, both compared to native BSA at the same residence time. Similar reductions in DBC were reported by Moosmann et al. [3] for 30 kDa PEG-lysozyme on several commercial cation exchangers. In that study, however, the effects of PEGylation were less pronounced at low pH and low salt, when protein binding was strong for both native and PEGylated lysozyme, but quite large at higher pH values, where binding of PEGylated lysozyme became very weak or non-existent. Although adsorption kinetics has been postulated to be the underlying reason for the dramatic effects of PEGylation on DBC, to the best of our knowledge direct measurements of adsorption isotherms and kinetics are lacking and only tenuous connections have been made for PEGylated proteins between adsorption kinetics and resin architecture. The kinetics of displacement of native and PEGylated proteins is also not completely understood. As noted by Fee and Van Alstine [6], when a mixture of native and PEGylated proteins is loaded onto a chromatography column, sample self-displacement can occur as a result of competition for the same binding sites. On the other hand, this effect is likely impacted by the adsorption kinetics, which, in turn, is influenced by both the molecular properties of native and PEGylated species and the physical properties of the stationary phase.

The objective of this work is thus two-fold. The first is to determine single-component adsorption equilibrium properties and binding kinetics for native and PEGylated proteins on two representative anion exchangers – one with a macroporous structure and the other based on the same backbone matrix, but with a polymer-grafted architecture. The second is to determine the multicomponent adsorption kinetics when native and PEGylated proteins are loaded either simultaneously or sequentially on these materials. BSA and BSA PEGylated with 10 and 30 kDa PEG chains are used as a model. Resin physical properties are characterized experimentally and adsorptive properties of native and PEGylated BSA are obtained through a combination of chromatographic, batch adsorption, and confocal laser scanning microscopy (CLSM) experiments.

2. Materials and methods

2.1. Materials

The anion exchange resins used in this work are UNOsphere™ Diol Q (hereinafter referred to as UNO Q) and Nuvia Q™, from Bio-Rad Laboratories (Hercules, CA, USA). Nuvia Q is a commercial material while UNO Q was prepared for this study for use as a reference material. Both resins are based on the same polymeric backbone and contain quaternary ammonium ion functional groups. However, UNO Q has an open pore structure while Nuvia Q contains grafted, charged polymeric surface extenders. Relevant properties of these materials are summarized in Table 1.

Bovine serum albumin (BSA) ($M_r \sim 66$ kD, $pI \sim 5$) with ≥98% purity based on agarose gel electrophoresis was obtained from Sigma–Aldrich (St. Louis, MO, USA, Type A7906). The sample was further purified by SEC using a Superdex 200 column from GE Healthcare (Piscataway, NJ, USA) to remove the BSA dimers and oligomers that were found present at a level of about 20% of the total protein. Maleimido-PEG reagents were obtained to produce PEG-BSA conjugates. Linear 10 kDa PEG-maleimido reagent was from Jenkem Technology (Beijing, China) while linear 30 kDa PEG-maleimido reagent was from NOF Corporation (White Plains, NY, USA). Chemicals for buffer preparation, Tris, BisTris-propane (BTP) and HCl were from Sigma–Aldrich (St. Louis, MO, USA) and Fisher Scientific (Pittsburg, PA, USA). For consistency with prior work [5], all protein concentrations reported in this work, both in solution