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Protein adsorption to poly(ethylenimine)-modified Sepharose FF: II. Effect of ionic strength

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ABSTRACT

In Part I of this work, we have studied the effect of ionic capacity (IC) on bovine serum albumin (BSA) adsorption equilibria and kinetics to poly(ethylenimine) (PEI)-grafted Sepharose FF, and found a critical IC (cIC, 600 mmol/L), above which both protein capacity and uptake rate increased drastically. In this work, five PEI-Sepharose FF resins of typical ICs reported earlier were selected to explore the effect of ionic strength (IS) on the adsorption equilibria and kinetics of BSA. Commercially available DEAE (IC = 160 mmol/L) and Q Sepharose FF (IC = 269 mmol/L) resins were used for comparisons. It is found that at similar ionic capacities, protein adsorption capacities on both the PEI-Sepharose FF resins and the commercial resins decreased with increasing IS, but on the capacity sensitivity to salt concentration, the former was lower than the latter. In addition, the effective diffusivities (D_e) of the former were smaller than the latter in the entire IS range studied. The low IS sensitivity of adsorption capacity of the PEI-Sepharose FF resins could be interpreted by the increase of pore accessibility with increasing IS; the smaller D_e values in the PEI-Sepharose FF resins were considered due to the lack of surface diffusion in the PEI-Sepharose FF resins of low PEI densities. For the PEI-Sepharose FF resins of high ICs (520, 740 and 1220 mmol/L), both protein capacity and D_e values increased first and then decreased with increasing IS. The increasing trend of protein capacity in the low IS range was considered due to the increase of accessible pores for BSA. The rise–fall trend of D_e was attributed to the dependencies of the "chain delivery" effect on protein capacity and binding strength, both of which are related to IS. Moreover, the IS sensitivity of the D_e for the resins of ICs > cIC (740 and 1220 mmol/L) was much higher than those of ICs < cIC, further proving that the "chain delivery" effect in PEI layer did contribute significantly to the overall mass transfer at IC > cIC. Furthermore, the two PEI-Sepharose FF resins of ICs > cIC kept high adsorption capacities and D_e values up to 200–300 mmol/L NaCl. Therefore, the operating IS ranges for these two PEI-Sepharose FF resins can be much broader than the traditional ion-exchange media.

1. Introduction

Ion-exchange chromatography (IEC) has been widely applied to protein purification in the downstream processes of biotechnology. To enhance IEC performance, ion-exchange media used for IEC should be designed to possess high dynamic binding capacity at high flow rates [1,2]. To this end, the media must have high equilibrium binding capacity as well as high mass transfer rate for protein adsorption. Recently, researchers have found that functionalized-polymer grafted ion exchangers show significantly improved adsorption capacities and uptake rates [3–7] as compared with traditional ion exchangers. Several mechanistic origins of these advantages associated with polymer-grafting have been proposed [5–10].

In Part I of this work [11], we have investigated the effect of ionic capacity (IC) on bovine serum albumin (BSA) adsorption to poly(ethylenimine) (PEI)-modified Sepharose FF. It was found that PEI modification greatly enhanced BSA adsorption capacity and uptake kinetics, when the grafting densities were higher than a critical IC (cIC, about 600 mmol/L). Comprehensive analysis suggests that in the high PEI coupling density range, where IC > cIC, the neighboring PEI chains become close enough to each other, and the chains are bonded to the resin surface with minimum number of coupling sites, so they become extended to the pore space, with greater flexibility and more accessibility for protein molecules. Consequently, above the cIC, a "chain delivery" effect driven by the chemical potential toward the bead center as well as the

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interactions between neighboring flexible chains mediated by bound proteins, facilitated protein transfer by the flexible chain swings. The "chain delivery" effect looks like a flexible chain with bound protein molecules interacts with a neighboring chain in the direction to the bead center and passes the protein molecules to the chain. This is similar to what Tao et al. discussed in antibody adsorption to Capto S [10]. Similarly, the enhanced adsorption capacity is attributed to the extended PEI layer, which provided a three-dimensional binding volume with good accessibility for protein binding [12,13].

Besides ionic capacity, ionic strength (IS) is another important factor influencing protein adsorption behaviors in IEC. Because IEC is based on electrostatic interactions, bound protein in IEC is generally eluted by increasing IS to a high value (e.g., 0.5–1.0 mol/L). In a low IS range, however, increasing IS weakens both protein–resin attraction and protein–protein repulsion, giving rise to the changes of "chain delivery" effect and "adsorption-caused hindrance" effect, which have been found to significantly contribute to protein adsorption in BSA adsorption to PEI-Sepharose FF [11]. Additionally, because PEI chains are highly charged at the binding condition, their shape and flexibility, which are also related to protein adsorption [11], may also heavily depend on IS.

Hence, this work was designed to investigate the effect of IS on BSA adsorption equilibria and kinetics to PEI-Sepharose FF resins. To this purpose, five PEI-Sepharose FF resins at typical IC values [11] were selected. Q Sepharose FF and DEAE Sepharose FF were used for comparisons and BSA was used as a model protein. The salt concentration range investigated herein was 0–500 mmol/L NaCl, broader than those reported previously in IEC (0–300 mmol/L) [9,12,14–19]. The research is expected to provide more insights into the role of grafted polymer chains on protein uptake and to help optimizing an IEC operation with PEI-grafted anion exchangers.

2. Materials and methods

2.1. Materials

DEAE Sepharose FF and Q Sepharose FF were purchased from GE Healthcare (Uppsala, Sweden). PEI (molecular weight of 60,000) grafted ion exchangers (FF-PEI-L160, FF-PEI-L260, FF-PEI-L520, FF-PEI-L660, FF-PEI-L740, and FF-PEI-L1220) used in this work were prepared with Sepharose FF as described in Part I of this work [11]. Their physical properties are listed in Table 1. BSA ($M_W \sim 66\,400$, p$I \sim 4.9$) was obtained from Sigma–Aldrich (St. Louis, MO, USA), and was directly used without removing dimer as described in Part I of this work [11]. Glucose and dextran standards for the inverse size exclusion chromatography (iSEC) were purchased from National Institute of Metrology (Beijing, China) and their average molecular weights (M_W) are listed in the Supplementary Material (Table S1). Tris(hydroxymethyl)aminomethane (Tris), sodium chloride (NaCl), and other reagents were of analytical grade from Sangon Biotech Co., Ltd. (Shanghai, China).

Protein solutions were prepared by dissolving in equilibration buffer (20 mmol/L, Tris–HCl, pH 8). The protein content was adjusted photometrically with a Lamda 35 UV/VIS spectrophotometer (Shelton, CT, USA) at 280 nm, using an extinction coefficients E^{mM} (280 nm) = 45.5 for BSA [20].

2.2. Inverse size exclusion chromatography

iSEC was used to determine the effect of salt concentration on the accessible pore size. The chromatographic experiments were conducted at varying NaCl concentrations in the equilibration buffer using the method described earlier [11]. The sample concentrations of glucose and dextran standards and their values of the viscosity radius (R_η) calculated using the equation $R_\eta = 0.0271 \times M_W^{0.498}$ [21] are listed in Table S1.

The distribution coefficient (K_D) at each IS was calculated according to the following equation,

$$K_D = \frac{V_R - V_0}{V_T - V_0} \quad (1)$$

where V_R is the peak retention volume, V_0 is the interparticle void volume determined by using dextran 521k for the PEI-Sepharose FF resins, and V_T is the total mobile phase volume determined by using glucose.

The modified single cylindrical pore model described in Part I of this work [11] was used to analyze the iSEC data and to give the apparent pore radius (r_{pore}).

In the above studies, each chromatographic experiment was conducted in triplicate and the average K_D value is reported. By fitting the modified single cylindrical pore model to the average K_D values, the r_{pore} value is determined with its standard error.

2.3. Adsorption isotherms and kinetics

The static adsorption experiments of BSA on the anion exchangers were respectively carried out at varying NaCl concentrations in equilibration buffer (20 mmol/L Tris–HCl, pH 8), with the same method described in Part I of this work [11]. The Langmuir model was used to describe the adsorption isotherms,

$$q = \frac{q_m c}{K + c} \quad (2)$$

where q_m is the adsorption capacity, K is the dissociation constant, c is the equilibrium liquid-phase concentration, and q is the equilibrium solid-phase concentration (based on mL of particle volume).

By fitting Eq. (2) to the isotherm data, q_m and K were determined with standard errors.

Dynamic uptake experiments were conducted in a 200-mL three-neck round-bottom flask at varying NaCl concentrations in the equilibration buffer (20 mmol/L Tris–HCl, pH 8) following the method described in Part I of this work [11]. Initial BSA concentration was 1 mg/mL. At one NaCl concentration, the mass of each adsorbent (m) was adjusted to the same. The effective pore diffusivity of the protein (D_e) was calculated by fitting the experimental uptake data to the pore diffusion model described earlier [11]. Because of the complex mass transfer mechanisms, D_e is a lumped kinetic parameter describing the overall uptake rate of protein adsorption. The free solution diffusivity (D_0) of BSA is 6×10^{-11} m^2/s at 25 °C [22].

In the above studies, each kinetic experiment was conducted in triplicate and the average value with its standard deviation is reported.

3. Results and discussion

3.1. Accessible pore size

Three PEI-Sepharose FF resins, FF-PEI-L520, FF-PEI-L660 and FF-PEI-L740, whose ionic capacities are around the cIC described earlier [11], and FF-PEI-L1220, were chosen to investigate the effect of NaCl concentration on the accessible pore size. The dextran calibration curves are shown in Fig. 1. It is obvious that the curves for FF-PEI-L520, FF-PEI-L660 and FF-PEI-L740 shifted to right with increasing IS, giving rising to smaller K_D values at each radius. While the curves for FF-PEI-L1220 were not affected by NaCl concentration. The modified single cylindrical pore model provided a good description for each one of FF-PEI-L520, FF-PEI-L660 and FF-PEI-L740 at each NaCl concentration as well. However, in order to have a clear view, the calculated K_D data are not given in Fig. 1, because