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Protein adsorption to poly(ethylenimine)-modified Sepharose FF: I. A critical ionic capacity for drastically enhanced capacity and uptake kinetics

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ABSTRACT

To explore the details of protein uptake to polymer-grafted ion exchangers, Sepharose FF was modified with poly(ethylenimine) (PEI) to prepare anion exchanger of 10 different ionic capacities (ICs, 100–1220 mmol/L). Adsorption equilibria and kinetics of bovine serum albumin (BSA) were then studied. It is found that ionic capacity, i.e., the coupling density of PEI, had significant effect on both adsorption capacity (q_m) and effective protein diffusivity (D_e). With increasing ionic capacity, the q_m value increased rapidly at IC < 260 mmol/L and then increased slowly till reaching a plateau at IC = 600 mmol/L. In the IC range of 100–600 mmol/L, however, the D_e values kept at a low level ($D_e/D_0 < 0.07$); it first decreased from 0.05 ± 0.01 at IC = 100 mmol/L to 0.01 ± 0.01 at IC = 260 mmol/L and then increased to 0.06 ± 0.01 at IC = 600 mmol/L. Thereafter, sharp increases of the q_m and D_e values [36% (from 201 to 273 mg/mL) and 670% (from 0.06 ± 0.01 to 0.49 ± 0.04), respectively] were observed in the narrow range of IC from 600 to 740 mmol/L. Finally, at IC > 740 mmol/L, the q_m value decreased significantly while the D_e value increased moderately with increasing the IC. The results indicate that PEI chains played an important role in protein adsorption and transport. In brief, there was a critical IC (cIC) or PEI chain density, above which protein adsorption and transport behaviors changed drastically. The cIC was identified to be about 600 mmol/L. Estimation of PEI grafting-layer thickness suggests that PEI chains formed an extended three-dimensional grafting-layer at IC > cIC, which provided high flexibility as well as accessibility of the chains for protein binding. Therefore, at IC > cIC, the adjacent PEI chains became close and flexible enough, leading to facilitated transport of adsorbed protein molecules by the interactions of neighboring chains mediated by the bound molecules. It is regarded as "chain delivery" effect. At the same time, improved accessibility of binding sites led the significant increase of binding capacity. The decrease of q_m value at IC > 740 mmol/L is considered due to the decrease of effective porosity. The research has thus provided new insight into protein adsorption and transport in polymer-grafted ion-exchange media.

1. Introduction

Ion exchange chromatography (IEC) has been playing the most important in the downstream processing of biologics, especially in the large-scale purification of proteins for pharmaceutical applications. In order to obtain better separation performance, polymer-functionalized IEC adsorbents, represented by dextran-grafted agarose gels [1–3], have been developed and widely studied for IEC. Compared with traditional IEC resins which ion-exchange groups are located on matrix surfaces, the polymer-grafted resins showed significant increases in dynamic binding capacities (DBC) of proteins [4–6], which reflects a significant improvement in both adsorption equilibrium and mass transport properties. Generally, high equilibrium binding capacity is attributed to the three-dimensional binding volume [7,8], and faster mass transfer is thought to be related to one or some of the following behaviors: the solid or homogeneous diffusion [4], shorter path for protein transfer [9], electrostatic coupling of diffusion fluxes [10], "bucket brigade" effect (protein passed from one polymer chain to the next) [11], and/or other effects existing in the grafting layer [12,13]. Technically, the "bucket brigade" effect can be regarded as "chain delivery" effect caused by the interactions of neighboring flexible chains, by which the bound proteins on one chain are transferred to the next.

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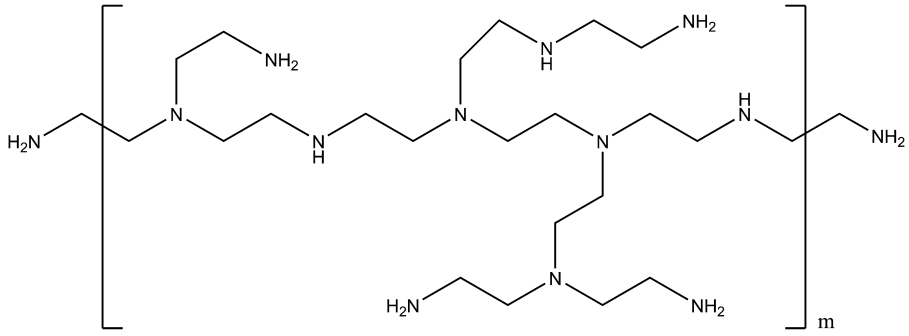

Fig. 1. Chemical structure of PEI.

To date, however, most efforts have been made on IEC resins prepared by grafting electro-neutral polymer chains [5,8,9,14–17] followed by coupling short ion-exchange groups. The post-grafting functionalization by ion-exchange groups makes the ionic groups exist in both the grafting layer and on the matrix surfaces, and their distribution may have impacts on protein uptake kinetics. Therefore, until now, the roles of the grafting layer on protein adsorption and transportation have not been independently studied in polymer-grafted resins.

By contrast to the IEC materials prepared by post-grafting functionalization by ion-exchange groups, little attention has now been paid to developing resins by directly grafting charged polymers [18]. This work is thus designed to develop an IEC material with a charged polymer, poly(ethylenimine) (PEI). PEI is a cationic polyelectrolyte with branched chains, containing primary, secondary and tertiary amino groups in a ratio of 1:2:1 [19] (Fig. 1). As a polymer of the highest charge density currently available (23.3 mequiv./g in aqueous solution when fully protonated) [20] and good biocompatibility, PEI has been widely used in the separation and purification of biologics, such as nucleic acid binding [21,22], endotoxin removal [23,24], heparin adsorption [25], and so on. Additionally, PEI was found to keep the activity and to enhance the stability of enzymes, so PEI was used as an ion-exchange ligand for enzyme immobilizations [26,27]. These applications demonstrated the possibility and benefits of using PEI in protein chromatography [18,28,29].

PEI has a similar structure with that of dextran, which is widely used in solid phase modifications for IEC. For example, both of them are macromolecules of long flexible chains. So, PEI-grafted resins have the possibility of keep the advantages of dextran-grafted resins in protein adsorption and transport. Meanwhile, some differences exist between PEI and dextran. PEI has more branches [19,30] than dextran (<5%) [31], and can be anchored at more than one point on a surface [18], resulting in less flexibility of the chains. Besides, the ionic group distribution in PEI-grafted resins, whose ionic groups only exist in the PEI layer, is different from that in dextran-grafted IEC resins. Therefore, the chromatographic performance of PEI-grafted resins would show some differences from dextran-grafted ion exchangers. Moreover, the effect of the grafting layer on protein uptake can be examined independently in PEI-grafted resins, excluding the influence of the ionic groups on matrix surfaces.

In this article, 10 IEC media of different PEI densities (ionic capacities) were synthesized and their adsorption equilibria and uptake kinetics were investigated using bovine serum albumin (BSA) as a model protein. The results are expected to provide new insights into the roles of grafted-polymer chains on protein uptakes and to benefit the selection and design of suitable media for IEC purification of therapeutic proteins.

2. Materials and methods

2.1. Materials

Sepharose FF was purchased from GE Healthcare (Uppsala, Sweden). PEI (50% (w/w) solution in water, 60,000 in number-averaged molecular weight (M_n), 750,000 in weight-averaged molecular weight (M_w), 12.5 in polydispersity index), blue dextran (M_W ~2,000,000) and BSA (M_W ~66,400, pI ~4.9) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Glucose and dextran standards used for the inverse size exclusion chromatography (see below) were obtained from the National Institute of Metrology (Beijing, China) and their average molecular weights are listed in Table 1. Dimethyl sulfoxide (DMSO) and epichlorohydrin (ECH) were of analytical grade from Guangfu Fine Chemical Research Institute (Tianjin, China). Sodium chloride (NaCl), tris(hydroxymethyl)aminomethane (Tris) and other reagents were of analytical grade from Sangon Biotech Co., Ltd. (Shanghai, China).

Protein solutions were prepared 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 coefficient of E^{mM} (280 nm) = 45.5 for BSA [32].

Heterogeneity analysis of BSA using size exclusion chromatography showed a dimer amount of about 8% (see Supplementary Material). Although the presence of dimer and/or oligomers could have effects on chromatographic separation behavior, these effects mainly existed in column operations, such as tailing in breakthrough curves [33]. Since no chromatographic separation occurred in the present batch adsorption experiments, the effect of the dimer is considered small [34]. Therefore, BSA sample in this work was used without removing the dimer.

2.2. Fabrication of PEI-grafted resins

PEI-grafted Sepharose FF resins were fabricated following the method described in our earlier work [35]. The grafting density

Table 1
Average molecular weights (M_w), viscosity radii (R_η), and sample concentrations of glucose and dextran standards used for iSEC experiments.

Dextran	M_w (Da)	R_η (nm)	Concentration (mg/mL)
Glucose	180	0.36	5.0
Dextran 4k	4320	1.75	5.0
Dextran 12.6k	12,600	2.99	4.0
Dextran 60.6k	60,600	6.53	3.0
Dextran 110k	110,000	8.78	2.0
Dextran 289k	289,000	14.2	1.2
Dextran 521k	521,000	19.1	0.9
Blue dextran	2,000,000	37.2	1.2