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Characterization of poly(allylamine) as a polymeric ligand for ion-exchange protein chromatography

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ABSTRACT

This work reports poly(allylamine) (PAA), as a polymeric ion-exchange ligand for protein chromatography. Sepharose FF was modified with PAA, and six anion exchangers with ionic capacities (ICs) from 165 to 618 mmol/L were prepared. Inverse size exclusion chromatography, adsorption equilibrium, uptake kinetics and column elution were performed. It was found that both the adsorption capacity and effective diffusivity maintained low values in the IC range of 165–373 mmol/L, but they started to increase beyond 373 mmol/L, and increased by 80% and 23 times, respectively, when the IC reached 618 mmol/L. Interestingly, a drastic decrease of pore size was observed around the IC of 373 mmol/L. The results suggest that the PAA chains played an important role in protein adsorption by altering the inner pore structure of the gels. It is considered that, PAA chains turn from inextensible states with multipoint-grafting on the pore surface at low coupling densities (IC<373 mmol/L) to closer, extended and flexible grafting states with less coupling points at higher coupling densities (IC>373 mmol/L). These characters of the grafted chains at higher IC values benefit in protein adsorption by three-dimensional binding and encouraged the happening of “chain delivery” of bound proteins on the chains. Besides, the ion exchangers showed favorable adsorption and uptake properties in a wide ionic strength range, 0–500 mmol/L NaCl, indicating much better salt tolerance feature than the so-far reported ion exchangers. Moreover, a mild condition of pH 5.0 offered effective recovery of bound proteins in elution chromatography. The results indicate that the PAA-based anion exchanger of a high IC value is promising for high-capacity protein chromatography dealing with feedstock of a wide range of ionic strengths.

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1. Introduction

Ion exchange chromatography (IEC), as a manageable and reliable technology, has been widely applied in the purification of proteins, antibodies and vaccines [1–3]. Compared with traditional IEC resins whose ion-exchange groups are only located on matrix surfaces, polymer-functionalized ion-exchangers, whose partial or even entire ion-exchange groups are located on polymer chains, have been demonstrated to offer markedly enhanced dynamic binding capacities (DBC) of proteins [4,5], indicating a significant improvement in both equilibrium adsorption capacity and mass transfer kinetics. Generally, the enhanced equilibrium adsorption capacity is considered due to the three-dimensional binding volume offered by the grafting layer [6,7], and the accelerated mass

transfer rate is attributed to “chain delivery” effect of bound proteins between grafted polymer chains [8,9].

To date, several strategies have been developed to fabricate polymer-grafted adsorbents. Initiating the polymerization of monomers from the matrix surface is an effective way to produce polymer chains with single-anchored sites “grown” on the matrix surface. This is called the “grafting from” process, represented by grafting glycidyl methacrylate to Sepharose 6 Fast Flow (Sepharose FF) gel to obtain the resins with extremely high capacities [10]. However, most of resins prepared by the “grafting from” strategy exhibited the absence of significantly increased uptake kinetics [11]. Moreover, the selection of monomer generally needs to balance the chemical reactivity (polymerization activity, potency of functionalization, and the complexity of polymerization) with biocompatibility. Hence, limited efforts have been made on polymer-grafted resins affording favorable uptake properties by the “grafting from” strategy [10].

Alternatively, polymer-grafted resins can be synthesized by directly grafting existing polymer chains to matrix surfaces, which

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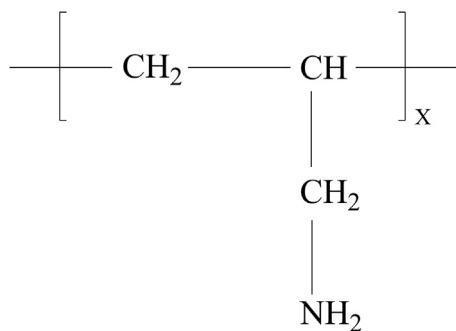


Fig. 1. Chemical structure of PAA.

is the “grafting to” strategy. Many excellent resins with favorable chromatographic performance were prepared by this strategy [12,13], as represented by dextran-grafted agarose gel (including the commercial Sepharose XL series) and poly(ethylenimine) (PEI)-grafted Sepharose resins [5,7,8,14,15]. For the grafting of electro-neutral polymer chains, a following step of coupling short ion-exchange groups is indispensable; the post-grafting and then functionalization makes the ionic groups exist in both the grafting layer and on the matrix surfaces, giving rise to heterogeneous binding sites. By contrast, direct grafting of polyelectrolytes would be more efficient [16].

Poly(allylamine) (PAA) is a linear polymer with pendant primary amino group per monomer unit, as its chemical structure shown in Fig. 1. As an alkaline polyelectrolyte ($pK_a = 9.67$) [17], PAA is positively charged at pH 6.0–8.0, which is the typical pH range of biological feedstock. Additionally, PAA possesses good biocompatibility, so it has been widely used in biochemical applications, such as vascular graft [18], protein adhesion [19], immunosensor [20], fluorescent nanocrystals [21] and antibiotic nanoparticles [22]. Recently, PAA has been reported as an ion-exchange ligand, and a PAA modified Sepharose FF resin, named C2c with ionic capacity (IC) of 669 mmol/L, exhibited higher DBC value than Q Sepharose FF at 0.25 mol/L NaCl concentration [23,24]. Therefore, the detailed protein adsorption and chromatographic behaviors with PAA-grafted ion exchangers are worthwhile studying for their further applications. Moreover, the fundamental mechanism of protein adsorption onto PAA-grafted resins, particularly the roles of PAA chains and the effects of salt concentration on protein adsorption equilibrium and kinetics, needs to be explored to better understand the properties of the materials. In addition, because of the high binding ability at high salt concentration reported previously [23], traditional IEC elution with high ionic strength (IS) solution may not be suitable. Hence, seeking a suitable elution condition becomes of significant importance for its application.

In this work, PAA was grafted on the Sepharose FF gel, and anion exchangers with six different ICs (PAA grafting densities) were synthesized. Using bovine serum albumin (BSA) as the model protein, the adsorption equilibria and uptake kinetics in the six PAA-grafted resins were investigated at ISs of 0–500 mmol/L NaCl. Column chromatography elution was conducted to investigate protein elution behavior at different pH values. The results are expected to help the understanding of the protein adsorption and elution behaviors on PAA-grafted resins, and provide insights into the roles of PAA chains on protein adsorption.

2. Materials and methods

2.1. Materials

Sepharose FF was purchased from GE Healthcare (Uppsala, Sweden). BSA (>96%, $M_w \sim 66,400$ Da, $pI \sim 4.9$, used as received,

without further purification), blue dextran ($M_w \sim 2,000,000$ Da) and poly(allylamine hydrochloride) (M_w range 12,000–22,500 Da, average $M_w \sim 17,500$ Da) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and epichlorohydrin (ECH) were of analytical grade from Guangfu Fine Chemical Research Institute (Tianjin, China). NaOH, NaCl, tris (hydroxymethyl) aminomethane (Tris) and other reagents were of analytical grade from Sangon Biotech Co., Ltd. (Shanghai, China).

2.2. Preparation of PAA-grafted Sepharose FF gel

The synthesis scheme of PAA-grafted Sepharose FF gel is illustrated in the Supplementary material (Fig. S1), similar to that reported previously for the coupling of PEI [8]. Briefly, 1 g of Sepharose FF was mixed with 2 mL of 1 mol/L NaOH, 1 mL ECH and 2 mL DMSO for 3 h in a water bath shaker at 25 °C and 170 rpm. This procedure leads to the gel activation with epoxy groups for further modification [25]. The ECH-activated agarose beads were then washed with distilled water to remove residual reagents (the washed water was detected by phenolphthalein- $\text{Na}_2\text{S}_2\text{O}_3$ solution) and drained in G3 glass filter. The drained activated gel (1 g) was suspended in 1 mL PAA solution by shaking at 170 rpm for 12 h to allow poly(allylamine hydrochloride) to diffuse into the particle pores. Thereafter, 50% (w/w) aqueous solution of NaOH was added into the suspension to adjust the pH to 12.5 for the initiation of PAA grafting reaction, and the slurry was shaken in the incubator at 25 °C and 170 rpm for 24 h. The product was washed with excess distilled water and the washed water was detected by phenolphthalein solution. Finally, The PAA-modified gels were then stored in 20% ethanol solution for further use. The PAA concentration in the coupling reaction was changed in the range of 2% to 40% (w/w) to obtain anion exchangers of different IC values.

2.3. Ion exchanger characterization

The ICs of the resins were determined by silver chloride precipitation titration following the method described by Yu et al. [8]. The size distribution and volume-weighted average diameter of resin particles (d_p) were measured with a Mastersizer 2000U particle size analyzer from Malvern Instruments (Worcestershire, UK). The density of the hydrated particles (ρ_p) was measured with a 25-mL pycnometer at 25 °C [8].

In the above measurements, each experiment was conducted in triplicate and the average value is represented.

2.4. Inverse size exclusion chromatography

Inverse size exclusion chromatography (iSEC) was used for the analysis of the accessible pore size, using the method described earlier [24]. The sample concentrations of glucose and dextran, as well as their values of viscosity radius (R_η) calculated from the equation of $R_\eta = 0.0271 \times M_w^{0.498}$ [26], are listed in Table S1.

All iSEC experiments were performed on an Agilent 1100 Series LC system with a refraction index detector (RID) and a 20- μL sample loop (Waldbronn, Germany) at varying NaCl concentrations in equilibration buffer (20 mmol/L Tris-HCl, pH 8.0), 25 °C. Each resin beads of Sepharose FF, FF-PAA-373, FF-PAA-532 and FF-PAA-618 were washed three times with the equilibration buffer plus 1 mol/L NaCl, and then loaded to a Tricorn 5/50 column (GE Healthcare, Uppsala, Sweden) by gravity settling. The column was then packed at 1.5 mL/min (450 cm/h) for at least three column volumes (CVs), producing a stable packed bed of 5.5 ± 0.2 cm in length (1.08 ± 0.04 mL in volume). The column efficiency was checked by acetone.

The column was equilibrated with 15 column volumes (CVs) of the solution and the detector was set to zero when the RID

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