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Fabrication of high-capacity cation-exchangers for protein chromatography by atom transfer radical polymerization

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ABSTRACT

This work reports the synthesis of novel cation-exchanger with controllable charge density and polymer chain length of poly(3-sulfopropyl methacrylate) (poly(SPM)) grafted via atom transfer radical polymerization onto Sepharose FF matrix. Polymer grafting provided a three-dimensional regular arrangement of the ligand and increased ionic capacity of the poly(SPM)-grafted cation-exchangers. The result showed that adsorption capacity for lysozyme enhanced greatly in poly(SPM)-grafted cation-exchangers whereas adsorption capacity for γ-globulin decreased dramatically. It can be attributed to the consequence of the competition between electrostatic interaction and repulsive excluded volume interaction of grafted polymer and protein. For lysozyme, protein adsorption on poly(SPM)-grafted cation exchangers was driven dominantly by electrostatic interaction. By constrast, the repulsive excluded volume interaction between grafted polymer and protein was remarkable in adsorption of γ-globulin on poly(SPM)-grafted cation exchangers. Moreover, the poly(SPM)-grafted cation exchangers exhibited great salt tolerance in protein adsorption and distinct intraparticle mass transfer properties. Finally, the results of dynamic binding capacity (DBC) for lysozyme showed that the poly(SPM)-grafted cation-exchangers had higher binding capacities than did the commercial SP Sepharose FF, and the maximal DBCs reached 192 mg/mL at 50 mmol/L NaCl. These results demonstrate great potential of poly(SPM)-grafted cation-exchangers for the large-scale purification of proteins.

1. Introduction

A bewildering variety of ion-exchange chromatographic adsorbents for the purification of proteins are currently available in the product catalogs of many manufacturers. Market data from GE Healthcare Bio-Sciences also showed that the ion-exchange chromatography (IEC) remained 45% of chromatographic shares for industrial purification [1]. In preparative IEC, binding capacity and throughput are the most important factors that must be considered. In recent decades, several innovative adsorbents for protein chromatography including biporous particles [2], superporous particles [3] and monoliths [4] have been developed to achieve a high binding capacity for proteins by alleviating the limitation of intraparticle mass transfer resistance [5]. However, this increased binding capacity often comes at the expense of the adsorption capacity. The binding capacity for proteins significantly depends on transport and kinetic events and is limited at the upper level by the adsorption capacity [6,7], which is a function of the protein size and the specific surface area of the adsorbents. In porous adsorbents that are commonly used for ion-exchange chromatography, the adsorbed protein is arranged in a loosely or densely packed monolayer on the pore surface. Therefore, the limited specific surface area is the major constraint to the adsorption capacity for proteins, especially for large proteins [7,8].

An effective way to improve the adsorption capacity for proteins is the utilization of the entire pore space of the adsorbents instead of just pore surface. Related techniques can be traced back to the advent of polymer composite ion-exchange adsorbents. Horvath et al. proposed a novel approach for the utilization of the pore space by filling functionalized polyacrylamide gel into the pores of a rigid polystyrene-silica material [9], and the resulting chromatographic material is well-known under the trade name HyperD™ adsorbents. HyperD ion-exchange adsorbents exhibited considerably higher adsorption and binding capacities for proteins than do conventional ion-exchange adsorbents [10,11]. Another important

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Table 1
Adsorption capacities of proteins on various polymer-grafted ion exchangers.

Protein	Ion exchangers	Grafted polymer	Buffer conditions	Adsorption capacity(mg/mL)	Refs
Lysozyme	SP-T40-X-S6B	40 kDa dextran	20 mmol/L NaCl in 10 mmol/L Na_2HPO_4 buffer (pH 6.5)	210	[18,19]
	SP-T10-X-S6B	10 kDa dextran		150	
	SP-D-SA Superporous gel with 6% agarose	40 kDa dextran	20 mmol/L NaCl in 3.9 mmol/L Tris–47 mmol/L Gly buffer (pH 8.2)	127	[20]
	SP Sepharose XL	dextran	10 mmol/L sodium phosphate buffer (pH 7)	203	[21]
	Streamline SP XL	dextran	50 mmol/L Glycine-HCl buffer (pH 9.5)	243	[22]
γ-globulin	SP Sepharose XL	dextran	1.3 mS/cm in 15 mmol/L acetate buffer	295 for Fab	[23]

approach that was proposed by Müller is to graft the polymer chains bringing ionic-exchange groups onto the pore surface of the matrix [12,13]. Polymer-grafted ion-exchange adsorbents present a distinct electrostatic interaction between the protein and the ligand, and provide a more complex pore surface of charged ligand over which the protein must diffuse [1]. However, the earliest polymer-grafted ion-exchange adsorbents, Fractogel™ EMD ion-exchanger, did not exhibit an advantage in binding capacity or intraparticle mass transfer events [14,15] until the advent of dextran-grafted ion-exchange adsorbents, as represented by Sepharose XL and Capto groups [16–18]. Dextran-grafted cation-exchangers are prepared by grafting and functionalizing the flexible dextran in agarose matrix and have shown higher adsorption capacities as listed in Table 1 [18–23] and more rapid intraparticle diffusivity than non-grafted cation-exchange adsorbents [17,18]. Recently, Yu et al. introduced charged polyethylenimine (PEI) directly to prepare PEI-grafted Sepharose FF and the anion-exchangers had maximum adsorption capacity of 273 mg/mL for bovine serum albumin [24,25]. They also found a critical ionic capacity in the PEI-grafted Sepharose FF, above which the protein adsorption capacity and effective diffusivity increased significantly [25]. Until now, several hypotheses and theoretical models have been developed based on considerable evidence that has accumulated over the last decade [18,24,26]. Because of multiple-point attachment and the wide distribution of molecular weight of polymers, the polymer layer in the polymer-grafted ion-exchangers mentioned above are intrinsically heterogeneous, and difficult to control and characterize [16]. Therefore, the adsorption and transport mechanism of protein in polymer-grafted ion-exchange adsorbents remains poorly understood.

In this paper, we reported the synthesis of novel cation-exchanger with controllable charge density and polymer chain length of poly(3-sulfopropyl methacrylate) (poly(SPM)) grafted via atom transfer radical polymerization (ATRP) onto Sepharose FF gel. Then, the static capacity and kinetics for lysozyme and γ-globulin adsorption of the synthesized poly(SPM)-grafted cation-exchangers were obtained in batch experiments. The results are discussed in detailed based on ionic capacity and apparent pore size. Finally, the dynamic binding capacities (DBCs) for lysozyme and γ-globulin of the poly(SPM)-grafted cation-exchangers were compared with those of the non-grafted SP Sepharose FF and the dextran-grafted SP Sepharose XL to evaluate the chromatographic performance of the poly(SPM)-grafted cation-exchangers.

2. Materials and methods

2.1. Material

Sepharose FF, SP Sepharose FF and SP Sepharose XL gels were purchased from GE Healthcare (Uppsala, Sweden). α-Bromoisobutyryl bromide (BIBB), 3-sulfopropyl methacrylate potassium and γ-globulin ($M_w \sim 150{,}000$, p$I \sim 6.5$) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Lysozyme ($M_w \sim 14{,}300$, p$I \sim 11.4$) was obtained from Genview (Houston, TX). Copper(I) bromide, Copper(II) bromide and 2,2′-Bipyridine (Bpy) were obtained from Dingguo Changsheng Biotech Co. Ltd (Beijing, China). Triethylamine (TEA), *N*,*N*-dimethyl formamide (DMF) and methanol were of analytical grade and were obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). Other reagents were of analytical grade from local suppliers and used without purification.

2.2. Fabrication of poly(SPM)-grafted cation-exchangers

2.2.1. Coupling of BIBB onto Sepharose FF gel

Poly(SPM)-grafted cation-exchangers were prepared by grafting SPM from Sepharose FF via ATRP, as illustrated in Fig. 1. Prior to grafting, BIBB was immobilized onto Sepharose FF gel in a 250-mL three-necked flask. After the storage buffer of the gel was substituted by washing with distilled water, DMF of increasing gradients (25%, 50%, 75% and 99.5% DMF in distilled water) and pure DMF, 15 g of drained Sepharose FF gel was transferred into the flask containing TEA (3.0 and 6.0 mL) in 100 mL of DMF. The mixture was degassed and cooled in an ice bath under nitrogen, and then 2.5 and 5.0 mL BIBB in 5 mL of DMF was added dropwise with stirring at 170 rpm to prepare two bromide- immobilized gels. The volumetric ratio of TEA to BIBB was 1.2:1. After BIBB was added in an ice bath, the mixture was left to warm at 30 °C and reacted continuously for 12 h. The resulting BIBB-immobilized gel was washed with excess DMF, ethanol, and distilled water in sequence. In this research, two bromide-immobilized gels with the low (2.5 mL) and high (5.0 mL) volumes of BIBB were prepared and called as Sep-BrL and Sep-BrH, respectively.

2.2.2. Grafting of SPM on bromide-immobilized gels

The ATRP of SPM on bromide-immobilized gels was carried out at room temperature to prepare poly(SPM)-grafted cation-exchangers. In a typical grafting process, 5 g of the bromide-immobilized gel was transferred into a 150-mL Erlenmeyer flask containing SPM in 50% (v/v) methanol aqueous solution (50 mL). After Bpy (312.36 mg, 2 mmol) and $CuBr_2$ (22.34 mg, 0.1 mmol) were added, the mixture was degassed under nitrogen at least for 30 min, and CuBr (143.45 mg, 1 mmol) was added to the flask. After the mixture was degassed again with nitrogen for 10 min, the flask was sealed and reacted under agitation at 170 rpm for 6 h. Then, the product was collected by filtering through a G3 sintered glass funnel, and residual copper ion was removed by immersing the product in excess 0.1 mol/L EDTA solution. Finally, the poly(SPM)-grafted cation-exchanger was washed with excess distilled water and stored at 4 °C for subsequent experiments. In this research, four