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Grafting glycidyl methacrylate to Sepharose gel for fabricating high-capacity protein anion exchangers

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ABSTRACT

To develop ion exchangers of high protein adsorption capacity, we have herein introduced atom transfer radical polymerization (ATRP) method to graft glycidyl methacrylate (GMA) onto Sepharose FF gel. GMA-grafted Sepharose FF resins of four grafting densities and different grafting chain lengths were obtained by adjusting reaction conditions. The epoxy groups on the grafted chains were functionalized by modification with diethylamine (DEA), leading to the fabrication of Sepharose-based anion exchangers of 14 different grafting densities and/or grafting chain lengths. The resins were first characterized for the effects of grafting density, chain length and ionic strength on pore sizes by inverse size exclusion chromatography. Then, the resins were evaluated by adsorption equilibria of bovine serum albumin (BSA) as a function of ionic capacity (IC) (chain length) at individual grafting densities. It was observed that at each grafting density there was a specific IC value (chain length) that offered the maximum equilibrium capacity. Of the resins with maximum values at individual grafting densities, the resin of the second grafting density with an IC value of 330 mmol/L (denoted as FF-Br2-pG-D330) showed the highest capacity, 264 mg/mL, about two times higher than that of the traditional ungrafted resin Q Sepharose FF (137 mg/mL). This resin also showed the most favorable uptake kinetics among the resins of similar IC values but different grafting densities, or of the same grafting density but different IC values. Effects of ionic strength showed that the capacities of FF-Br2-pG-D330 were much higher than Q Sepharose FF at a wide range of NaCl concentrations (0–200 mmol/L), and the uptake rates of the two resins were similar in the ionic strength range. Therefore, the dynamic binding capacity values of BSA on FF-Br2-pG-D330 were much higher than Q Sepharose FF as demonstrated at different residence times and ionic strengths. Taken together, the research has proved the success in the fabrication of high-capacity protein anion exchangers by grafting GMA onto Sepharose gel.

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1. Introduction

Ion exchange chromatography (IEC) is a well-established technology and has been widely applied in the purification of proteins, antibodies and vaccines [1–3]. As one of the mature chromatographic separation techniques for the large-scale purification of biologics, IEC still has a broad space for development and the exploitation of new ion exchangers continues to be one of the most important research directions [4–6]. Recent studies have found that functionalized-polymer grafted ion exchangers show many advantages in proteins adsorption over traditional ion exchangers, such as significantly improved adsorption capacities [7–9], which is attributed to the three-dimensional binding volume [10]. In addition, the polymer-grafted resins have been reported to achieve higher dynamic binding capacity (DBC) [11,12], which is one of the most important factors that must be taken into consideration in industrial production of biologics.

As efficient chromatographic media, polymer-grafted resins have been developed using various techniques. Some of them were fabricated on the basis of a "grafting to" process [13] by introducing polymer chains onto matrices, and showed favorable chromatographic performance, as represented by dextran-grafted agarose gels [11,14] (including the commercial Sepharose XL series) and poly(ethylenimine) (PEI)-grafted Sepharose resins [15]. However, the degree of branching and the molecular weight distribution of the polymers were heterogeneous, leading to the polymers chains located on matrix surface at heterogeneous states. In addition, generally, the polymer chains possess many reactive groups, so

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they could be randomly grafted onto matrix surfaces via multiple-point coupling, which aggravated the heterogeneity. This is the case for PEI-modified Sepharose gel, which a cationic polyelectrolyte of 12.5 in polydispersity index with indeterminate distributed branches [15]. Moreover, the heterogeneity of the added polymers by "grafting to" was indeterminate and would affect chromatographic behaviors. Therefore, the added polymers through "grafting to" led to a tendency to complicate synthetic issues of the resins, and then it was difficult to assess their effects on chromatographic behaviors [10].

As alternatives, polymer-grafted resins have been synthesized by "grafting from" strategies, by which the polymer chains "grow" from the initiator on the surface by polymerization of monomers. The "grafting from" strategies can generally bring out single-anchored site and homogeneous polymer chains. Specifically, of the "grafting from" strategies, surface-initiated atom transfer radical polymerization (SI-ATRP) [16,17] is one of the most powerful and versatile controlled radical polymerization techniques because its polymerization conditions and parameters can be readily tuned. When ATRP was used for the immobilization of polymers, the grafting density and grafting length could be tailored by altering the sacrificial initiator-to-monomer ratio as well as reaction time [18]. So the polymer chains locate on the matrix surface at homogeneous state, and the differences in grafted polymers are their grafting densities and chain lengths, which can be precisely controlled. The application of ATRP in the preparation of polymer-grafted ion exchangers was first reported by Ender et al. [19]. More recently, SI-ATRP has attracted much attention in the fabrication of polymer-grafted ion exchange adsorbents [20–22], and the resins showed improved characteristics in protein adsorption. Particularly, as reported by Yan et al. [21], poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) grafted magnetic polymer submicrospheres exhibited a high binding capacity of BSA up to over 660 mg/g.

In this study, we have attempted to develop high-capacity protein anion exchangers by grafting glycidyl methacrylate (GMA) to Sepharose FF gel using SI-ATRP and post-modification with diethylamine (DEA). In order to achieve resins of high protein adsorption capacities, GMA-grafted DEA-modified Sepharose FF resins of four grafting densities and different grafting chain lengths (ionic capacities) were prepared, leading to the fabrication of Sepharose-based anion exchangers of 14 different grafting densities and/or grafting chain lengths. Their adsorption equilibria and uptake kinetics were investigated using bovine serum albumin (BSA) as the model protein. The resin of highest static adsorption capacity was chosen to explore the effect of ionic strength (IS) on adsorption equilibria and kinetics, and to study the effects of residence time and IS on breakthrough behaviors. The research is anticipated to provide an efficient and controllable approach to the synthesis of high-capacity protein ion exchangers.

2. Material and methods

2.1. Materials

Sepharose FF was purchased from GE Healthcare (Uppsala, Sweden). Glycidyl methacrylate (GMA), bromoisobutyryl bromide (BIBB) 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 (iSEC) were products of the National Institute of Metrology (Beijing, China) and their average molecular weights were listed in Table S1. *N,N*-Dimethylformamide (DMF) and diethylamine (DEA) of guaranteed reagent and triethylamine (TEA) of analytical grade were from Guangfu Fine Chemical Research Institute (Tianjin, China). Tris(hydroxymethyl) aminomethane (Tris), sodium chloride (NaCl), 2,2′-bipyridine (Bpy) and other reagents of analytical grade were brought from Sangon Biotech Co., Ltd. (Shanghai, China).

Protein solutions were prepared in equilibration buffer (20 mmol/L Tris–HCl, pH 8.0) with different concentrations of NaCl. The protein concentration 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 [23].

2.2. Fabrication of GMA-grafted DEA-modified resins

The GMA-grafted ion-exchange resins were prepared by three steps, i.e., initiator immobilization on the gel surface, grafting GMA onto initiators via SI-ATRP, and modifying epoxy groups with DEA, as schematically illustrated in Fig. S1.

Prior to grafting, BIBB was immobilized on Sepharose FF surface as initiators for the SI-ATRP reaction. Generally, 6 g of drained Sepharose FF resins was soaked into 40 mL DMF containing 2.4 mL TEA, followed by dropwise addition of BIBB at 0 °C. Four different values of BIBB were used to change the initiator density on the matrix, as shown in Table 1. The mixture was gently stirred at 0 °C for 30 min and then the temperature was raised to 30 °C and reacted for 12 h. The BIBB-immobilized Sepharose FF (denoted as FF-Br) was washed thoroughly with DMF, ethanol and deionized water and stored at 4 °C for next grafting. The FF-Br gels prepared by addition of 0.15, 0.40, 0.64 and 2.00 mL of initial BIBB (Table 1) are referred to as FF-Br1, FF-Br2, FF-Br3 and FF-Br4, respectively.

The SI-ATRP of GMA onto the FF-Br gels was carried out using a reaction mixture of Bpy (156.2 mg, 1 mmol), $CuBr_2$ (5.6 mg, 0.025 mmol), drained FF-Br (6 g) and GMA in 50 mL of DMF and deionized water solution prepared in equal volume proportion. The initial GMA concentrations in the synthesis are listed in Table 1. This reaction mixture was stirred and degassed with high pure nitrogen for 30 min and then CuBr (35.86 g, 0.25 mmol) was added to initiate the reaction, which continued for 2 h at 25 °C. The GMA-grafted Sepharose FF, denoted as FF-Br-pG, was cleaned with excess ethanol and deionized water and kept at 4 °C before next processing.

The epoxy groups of GMA present on the FF-Br-pG resins were modified into amino groups by reacting in an aqueous solution of DEA (20%, v/v) for 12 h at 25 °C. The DEA was of excessive amounts for complete functionalization of the grafted GMA by amine groups. The GMA-grafted DEA-modified resins, denoted as FF-Br-pG-D, were extensively washed with deionized water and kept at 4 °C for using in the subsequent experiments.

2.3. Characterization of resins

The ion exchange capacities (IC) of the resins were determined by silver chloride precipitation titration following the method described by Yu et al. [15]. The volume-weighted average diameters of the particles (d_p) were measured with a Mastersizer 2000U particle size analyzer from Malvern Instruments (Worcestershire, UK). The densities of hydrated resins (ρ_p) were measured with a 25-mL pycnometer at 25 °C.

The bromine contents (grafting densities) of the resins were determined using oxygen bomb combustion-ion chromatography. Briefly, a known mass of sample was combusted for 30 min and then thoroughly rinsed with 25 mL of Na_2CO_3 (4.5 mmol/L)/$NaHCO_3$ (2.1 mmol/L) solution. The resulting brominated solution was analyzed by ion chromatography using a Thermo ICS1100 ion-chromatograph (Thermo Fisher Scientific, USA) fitted with an Ionpac AS22 column (Ø4 mm × 250 mm). The bromine content was calculated by the peak area identified as bromide at the chromatogram of the resin.

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