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Multilayer affinity adsorption of albumin on polymer brushes modified membranes in a continuous-flow system

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ABSTRACT

Polymer brushes modified surfaces have been widely used for protein immobilization and isolation. Modification of membranes with polymer brushes increases the surface concentration of affinity ligands used for protein binding. Albumin is one of the transporting proteins and shows a high affinity to bile acids. In this work, the modified membranes with cholic acid-containing polymer brushes can be facilely prepared by the immobilization of cholic acid on the poly(2-hydroxyethyl methacrylate) grafted microporous polypropylene membranes (MPPMs) for affinity adsorption of albumin. ATR/FT-IR and X-ray photoelectron spectroscopy were used to characterize the chemical composition of the modified membranes. Water contact angle measurements were used to analyze the hydrophilic/hydrophobic properties of the membrane surface. The modified MPPMs show a high affinity to albumin and have little non-specific adsorption of hemoglobin. The dynamic binding capacity of albumin in the continous-flow system increases with the cycle number and feed rate as the binding degree of cholic acid is moderate. The highest binding capacity of affinity membranes is about 52.49 g/m^2 membrane, which is about 24 times more than the monolayer binding capacity. These results reveal proteins could be captured in multilayers by the polymer brushes containing affinity ligands similar to the polymer brushes containing ion-exchange groups, which open up the potential of the polymer brushes containing affinity ligands in protein or another components separation. And the cholic acid containing polymer brushes modified membranes has the promising potential for albumin separation and purification rapidly from serum or fermented solution in medical diagnosis and bioseparation.

1. Introduction

Polymer brushes refer to polymer chains tethered to a surface or interface with a sufficiently high grafting density [1]. Strong exclusion forces make the polymer brushes adopt a unique stretched conformation and possess many distinctive properties [2–4]. There is a wealth of evidence to suggest that polymer brushes are very attractive platforms to bind proteins, which makes them of interest as tools for protein purification [2,5–7]. Compared with the self-assembled monolayers, the grafted polymer brushes can be considered as three-dimensional structures with a significant internal volume. As a consequence, polymer brushes present a very high surface concentration of affinity ligands that can be used for protein binding.

Protein binding to polymer brushes is either via nonspecific interactions [8,9], such as hydrophobic and electrostatic interactions, or via specific interactions [3,6,10], such as metal-affinity and receptor-ligand interactions. It has been demonstrated that the multilayer proteins were bound and the binding capacity was enhanced via electrostatic interactions [11,12] and metal-affinity interactions [13–16], where the binding occurs both at the brush surface and inside the brushes [2]. Lots of affinity binding of protein on the polymer brushes primarily focuses on the biotin-streptavidin interaction [17–19]. Other receptor-ligand interactions, such as sugar-lectin interactions [20–22], were also utilized for affinity adsorption of proteins. However, lots of receptor-ligand interactions existing in the natural world have not been exploited in the polymer brushes platforms for protein purification. As well known, protein purification through specific interactions can produce products with high purity in a simple process. Especially the affinity separation of the label-free protein from complex solutions, like fermented liquids and extraction solution, are in urgent need of development in the industries of biotechnology, medicine, and food.

Bile acids are natural compounds synthesized in the hepatocytes of the liver during the catabolism of cholesterol. About 95%

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of the primary bile acids are reabsorbed and sent to the liver for reuse by the portal vein, circulating in plasma bound to albumin with relatively high affinity [23,24]. Albumin is one of the transporting proteins, which is the most abundant blood protein and the most used protein in the clinical applications [25]. The high affinity that albumin shows for bilirubin, fatty acids, and certain organic dyes has led to the use of these compounds as ligands coupled to Sepharose and cryogels for the purification of albumin via static adsorption or bead chromatography [26–29].

In comparison to affinity bead chromatography, membrane chromatography offers some advantages, including higher flow rates, faster binding rates, lower pressure drop, higher productivity, and easier scale-up [21,30,31]. However, the main disadvantage of membrane chromatography for protein adsorption is a low binding capacity [32]. The introduction of polymer brushes with affinity ligands on membrane surface could provide a strategy to enhance binding capacity of protein [33]. Until now, little attention has been attempted on affinity separation of albumin with polymer brushes. Herein, it has been considered that proteins were captured in a monolayer at most by the polymer brushes containing affinity ligands [34].

In this work, cholic acid-containing polymer brushes were grafted on microporous polypropylene membranes (MPPMs) to construct affinity membrane for albumin separation. Hydrophilic poly(2-hydroxyethyl methacrylate) (poly(HEMA)) brushes were grafted on MPPMs at first. Then cholic acid molecules were bound on the grafted poly(HEMA) brushes via the reaction between the carboxyl groups of cholic acids and the hydroxyl groups of poly(HEMA). By taking the advantage of high porosity of MPPMs and high grafting degree (GD) of poly(HEMA), the binding degree (BD) of cholic acid can be controlled. These cholic acid-containing polymer brushes modified membranes are hopeful to be used as affinity membranes for the highly specific separation of albumin with high binding capacity in a continuous-flow system, supplying a new tool for albumin purification and separation from serum or fermented solution.

2. Experimental

2.1. Materials and chemicals

Commercial MPPM (Membrana GmbH, Germany) used in the experiments was prepared by a thermally induced phase separation method with a relatively high porosity about 75%. This membrane was cut into rotundity with a diameter of 25 mm and washed with acetone for 2 h to remove additives and impurities on the membrane surface. After drying under reduced pressure at 40 °C to constant weight (m_0), the MPPMs were weighted with an analytical balance (METTLER TOLEDO) to a precision of 0.01 mg and stored in a desiccator. HEMA (Sigama-Aldrich, 98%), benzophenone (BP), $FeCl_3 \cdot 6H_2O$, cholic acid, 4-dimethylaminopyridine (DMAP), N, N'-dicyclohexylcarbodiimide (DCC), acetone, and tetrahydrofuran (THF) were used without purification. BSA, bovine erythrocytes hemoglobin, and FITC-BSA solution (Sigma-Aldrich) were purchased and used as received. Water used in all experiments was de-ionized and ultrafiltrated to 18 MΩ with a Milli-Q system (Merck Millipore).

2.2. Cholic acid-containing poly(HEMA) brushes grafted on MPPMs

First, poly(HEMA) brushes were grafted onto the MPPMs by UV-induced grafting polymerization as described in our previous work [35]. Briefly, BP, $FeCl_3$, and HEMA were dissolved in acetone/water (1/1). Thereafter, a piece of MPPM was dipped in the solution for 60 min and then put into a UV processor, which was equipped with two 300 W high-pressure mercury lamps. After 10 min of purging with nitrogen gas, UV irradiation was carried out under nitrogen gas environment. Finally, the modified membrane was washed with acetone for 4 h and then with pure water for 20 h by vibration to remove any homopolymer and adsorbed monomer. After being dried in a vacuum oven at 40 °C to constant weight (m_1), the membrane was weighed with an analytical balance to a precision of 0.1 mg. The grafting degree (GD) of HEMA on the MPPM was defined as:

$$GD = \frac{m_1 - m_0}{130S} \quad (1)$$

where S is the surface area of the membrane. Each presented value was averaged from three parallel experiments.

Then, cholic acid was chemically bound to the poly(HEMA) brushes. The poly(HEMA) brushes grafted MPPM was dipped into THF in a round bottom flask. After that, cholic acid, DCC, and DMAP were added. The flask was sealed and shaken on a vibrator by 24 h at room temperature. Finally, the membrane was washed with ethanol for 4 h and then with pure water for 20 h by vibration. After being dried in a vacuum oven at 40 °C to constant weight (m_2), the membrane was weighed with an analytical balance to a precision of 0.1 mg. The binding degree (BD) of cholic acid on the MPPM was defined as:

$$BD = \frac{m_2 - m_1}{390S} \quad (2)$$

Each presented value was averaged from three parallel experiments.

2.3. Membrane characterizations

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FT-IR) measurement was carried on a Vector 22 FT-IR (Brucker Optics, Switzerland) equipped with an ATR cell (KRS-5 crystal, 45°). Sixteen scans were taken for each spectrum at a resolution of 2 cm^{-1}. Spectra of X-ray photoelectron spectroscopy (XPS) were recorded on a PHI-5000C ESCA system (Perkin-Elmer, U.S.A.) with Al Kα excitation radiation (1486.6 eV). The pressure in the analysis chamber was maintained at 10^{-6} Pa during measurements. All survey and core-level spectra were referenced to the C 1 s hydrocarbon peak at 284.7 eV in order to compensate for the surface charging effect. Scanning electron microscopy (SEM) images were taken on a Field Emission SEM (SIRION, FEI, USA) after the sample were coated with a 20 nm gold layer.

A CTS-200 contact angle system (Mighty Technology Pvt Ltd, China) was used for the determination of water contact angles at room temperature in air. Static contact angles were measured by the sessile drop method. First, a 1 μL drop of water was set onto the dry membrane surface with a microsyringe. Digital images for the droplet were then recorded. Contact angles were calculated from these images with software. Each reported value was an average of at least five independent measurements.

2.4. Static adsorption of protein

Static protein adsorption assays were performed to evaluate the recognition capability of the cholic acid-containing polymer brushes modified MPPMs. Briefly, membranes with a diameter of 5 mm were dipped into ethanol for 10 min. Then, the samples were washed with phosphate buffer solution (PBS, pH 7.36, 0.01 M) to exchange ethanol for 2 h. Subsequently, the membranes were immersed into 200 μL of FITC-BSA solution with a concentration of 0.02 mg mL^{-1} in PBS, and incubated at 25 °C for 2 h. The membranes were washed with PBS 6 times, each time using 200 μL buffer solution for 10 min. After being dried under vacuum at room