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Tetrazole-functionalized cation-exchange membrane adsorbers with high binding capacity and unique separation feature for protein

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ABSTRACT

The modification with high-density functional groups is a widely used method to enhance the binding capacity of membrane adsorbers, where the types of the attached groups are crucial to achieve good selectivity for protein separation. In this work, a novel tetrazole-functionalized weak cation-exchange membrane with high-capacity was prepared by constructing tetrazole-containing polymer brushes on the surface of regenerated cellulose membrane via the combination of surface-initiated atom transfer radical polymerization (SI-ATRP) and "click chemistry" between cyano and azide. Densities of tetrazolyl groups on the membranes can be easily controlled by manipulating the polymerization time. The binding capacity of lysozyme increased with the polymerization time but eventually reached maximum due to the reduction of the utilization percentage of ion-exchange sites. The adsorption of newly designed membrane conforms to the feature of weak cation exchanger in terms of pH and salt effects, whereas the effect of pH exhibits a large difference from that on the carboxylic-functionalized ion-exchange membranes. Most importantly, the membranes possess higher dynamic binding capacity independent of the flow rate of mobile phase compared with the previously reported cation-exchange membranes. Featured with the unique properties, the modified membrane could simultaneously purify lysozyme and ovotransferrin from hen egg white at higher productivity. The present work provides a new alternative for membrane chromatography of biomacromolecules.

1. Introduction

Membrane chromatography, which uses stacks of microporous membranes as stationary phase, is a potential method in the protein separation. In membrane-stacked column, convective mass transport can significantly raise the mass transfer rate [1]. As a result, membrane chromatography usually achieves a rapid protein separation rate via high flow-rate so as to approach high protein productivity per unit of time. Moreover, scale-up during the production of the therapeutic proteins can be conducted easily for membrane chromatography [2–3]. Consequently, it is of important value to study membrane chromatography of protein [4–6].

Despite the rapid separation rate, membrane chromatography has rather lower protein loading in each chromatographic cycle due to the low protein binding capacities of membrane, limiting improvement of productivity [1]. To enhance protein binding capacities of membrane, the polymer-brush structure, which can provide a three-dimension binding layer, has been proposed in the surface modification of membrane [7]. Muller is the first to graft polymer brushes onto the support surface to construct three-dimension binding layer, demonstrating the important role of polymer brushes on improvement of protein binding capacity [8]. Following this idea, several methods are developed to anchor the polymer brushes on the membrane surface such as photo-induced polymerization [9–10], radiation-induced polymerization [11–12] and surface-initiated ATRP (SI-ATRP) [13–15]. As a controlled/living polymerization method, SI-ATRP possesses high density and controllable chain-length of polymeric chains, and thus attracts much attention in the modification of membrane adsorbers [13–15].

As is well known, ion-exchange chromatography (IEC) is frequently employed in the protein separation because IEC can provide a gentler environment for active proteins. In particular for weak cation or anion exchange chromatography, a selective separation of the target protein can be easily achieved by optimizing pH of the mobile

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phase [16]. As for the preparation of ion-exchange membrane, the purpose of surface modification is to attach various charged groups at a high density as possible onto the membrane surface. Many works have demonstrated that SI-ATRP can endow the adsorbent surface with high-density functional groups, giving high protein binding capacity [17–22]. Our group also conducted some research works in this aspect [23–26]. Based on the types of the attached ion-exchange groups, the membrane adsorbers are usually divided into carboxylic, sulfonic acid, secondary ammonia or tert-ammonia, and quaternarium ammonium-type, on which the separation properties of protein have been investigated extensively. However, because of the complexity of biosamples in biotechnology, the currently available ion-exchange membranes are not able to isolate the target protein in some cases. Therefore, it is heavily desirable to solve this problem by developing new-type membrane.

H-tetrazoles possess the similar acidity and planarity as carboxylic [27]. In medicinal chemistry, H-tetrazoles often act as excellent bioisosteres to replace carboxylic in pharmaceutical molecular to develop new medicines [28–29]. Taking advantage of the acidity of H-tetrazole, we first developed a weak cation-exchange silica-based stationary phase [30]; The stationary phase showed excellent performance for protein separation with high mass recoveries and long-term stability. We also prepared tetrazole-functionalized fiber and resin for adsorption of heavy metal ions [31–32]. Since then, various tetrazole-functionalized adsorbers have been developed recently by other groups [33–39]. However, to the best of our knowledge, tetrazole-functionalized weak cation-exchange membrane has not been prepared yet, partly because of the commercial unavailability of the tetrazole ligands suitable to modify the materials [40].

Given that regenerated cellulose (RC) membranes are a commonly used matrix to develop various membranes, we attempted to take advantage of SI-ATRP to create a high-capacity tetrazole-functionalized weak cation-exchange RC membrane (TZ-RC). The procedure involved the grafting of polyacrylonitrile from RC membranes via SI-ATRP and the subsequent conversion of cyano into tetrazolyl groups via “click chemistry”. The properties of TZ-RC were investigated in details, and TZ-RC was used to purify lysozyme and ovotransferrin from hen egg white to show its unique separation selectivity.

2. Experimental section

2.1. Preparation of tetrazole-functionalized RC

2.1.1. Grafting of poly(acrylonitrile) (PAN) from the initiator-modified membranes

Initiator-modified membranes were obtained with the reaction of RC membranes and 2-bromoisobutyryl bromide according to our previous work [23].

CuBr (0.2 g), 2,2′-bipyridyl (0.4 g) and the initiator-modified membranes were placed into a 100-mL flask. High-purity nitrogen was bubbled to exchange the air. Then, the mixed solution of ethylene carbonate (40 g) and acrylonitrile (12.5 mL) was injected into the flask. Next, the flask was deoxygenated by three cycles of alternating freeze-pump-thaw and introduction of high-purity nitrogen. The reaction proceeded at 70 °C under a nitrogen atmosphere. Then, the PAN-grafted membranes were immersed in methanol-EDTA (1:1, V/V) and shaked for 10 min. Then, the PAN-grafted membranes were rinsed with water and ethanol for three times, and finally were dried at 40 °C until a constant weight was reached.

The grafting degree (DG) was calculated according to the following Eq. (1):

$$DG = \frac{m_{PAN} - m_{Br}}{m_{Br}} \times 100\% \tag{1}$$

where, m_{Br} and m_{PAN} are the mass of the initiator-modified and PAN-grafted membranes, respectively.

2.1.2. Preparation of tetrazole-functionalized membranes (TZ-RC)

The PAN-grafted membranes, NaN_3 (3.2 g), NH_4Cl (2.7 g) and DMF (50 mL) were placed into a flask. The reaction proceeded with gentle stirring at 100 °C for 16 h. Finally, the membranes were cleaned with ultrapure water to obtain the TZ-RC.

2.2. Water flux

The same filtration system and the same procedure as in the reference were used to assess the water flux of membranes [41]. The water flux was calculated by Eq. (2) and was measured three times to achieve the average value.

$$J = \frac{V}{At} \tag{2}$$

where, J is water flux (L/m^2/h), V is water volume (L), A is area of membrane (m^2), and t is filtration time (h).

2.3. Determination of protein binding capacity

2.3.1. Static binding capacity

Membranes with a given volume (V) were cut into pieces and soaked in 20 mM phosphate buffer solution (PBS, pH 7.0) for 5 min. Then, the membrane pieces were transferred into a given volume (V_0) of lysozyme with a given concentrations (C_0) and incubated for 16 h at 30 °C. The equilibrium concentrations of protein (C_e) were measured with ultraviolet spectrophotometer at 280 nm. The binding amount of lysozyme, Q, was calculated by Eq. (3),

$$Q = \frac{(C_0 - C_e) \times V_0}{V} \tag{3}$$

The adsorption isotherms were drawn by plotting Q against C_e, and were fitted by the Langmuir Eq. (4):

$$\frac{C_e}{Q} = \frac{C_e}{Q_m} + \frac{1}{K_L Q_m} \tag{4}$$

where K_L is equilibrium constant (mL/mg), Q_m is maximum binding capacity (mg/mL).

2.3.2. Dynamic binding capacity

A glass column was stacked with six membrane discs and connected to a HPLC instrument (LC-10AVP, Shimadzu, Japan). PBS initially equilibrated the column for 30 min, and then 3.0 mg/mL lysozyme in 20 mM PBS ran through the column at a given flow-rate to record the breakthrough curves at 280 nm. The dynamic binding capacity, $Q_{10\%}$, was calculated using the volume ($V_{10\%}$) at the 10% height of breakthrough curve by the following Eq. (5):

$$Q_{10\%} = \frac{3.0 \times V_{10\%} - V_d}{V} \tag{5}$$

where, V_d is the system dead volume (mL), and V is the membrane volume (mL).

2.4. Protein purification using TZ-RC membrane

Fresh hen egg white was diluted by 20 mM PBS (pH 7.0) in volume ratio of 1:10, and stirred vigorously for 30 min. The mixture was centrifuged at 20000 rpm under 4 °C for 20 min; the supernatant was filtered with 0.45 μm membrane prior to use.

The filtered supernatant was purified on the same equipment as in the dynamic binding capacity measurement, in which the column was packed with the membrane with grafting time of 16 h. The loading

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