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Polyacrylonitrile-based nanofibrous membrane with glycosylated surface for lectin affinity adsorption

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ABSTRACT

Affinity membrane chromatography is of great importance for the separation and purification of proteins. Electrospun nanofibrous mats decorated with the glycosylated surfaces, combining the merits of large surface area and high porosity as well as good specificity with lectin, are regarded as superior affinity membranes. We report a simple and effective approach to acquire a new kind of glycosylated nanofibrous membrane (GNM). Poly(acrylonitrile-*co*-hydroxyethyl methacrylate) (PAHM) was electrospun into nanofibrous mat with an average fiber diameter of 200 nm and glucose ligands were bound on the nanofiber surface through a reaction between glucose pentaacetate and the hydroxyl groups of PAHM. Interactions between the GNM surfaces and proteins were studied by confocal laser scanning microscopy. Static and dynamic protein adsorptions were also evaluated. Results indicates that the GNM selectively recognizes lectin concanavalin A (Con A) while shows almost no affinity binding with another lectin peanut agglutinin (PNA). It also shows strong multivalent and reversible binding capability as well as high adsorption capacity to Con A. We suggest that the GNM is promising in affinity chromatography for the separation and purification of lectins.

1. Introduction

Compared with traditional column chromatography, affinity membrane chromatography has attracted considerable attention in recent years for bioseparation due to its unique characteristics such as low pressure drop, fast transport rate, high binding efficiency, and easy scale-up [1–4]. Affinity membrane is a separation matrix immobilized with available ligands and plays a crucial role in corresponding chromatography. An ideal matrix for affinity membrane should possess reactive groups for further coupling of functional ligands, porous structure with physicochemical stability, and hydrophilic/biocompatible surface to prevent the nonspecific binding of proteins [1]. At present, a series of synthetic and natural polymers have been studied as matrices to fabricate affinity membranes for protein separation. Synthetic polymers [5–7] such as nylon and polysulfone have good mechanical properties while the nonspecific adsorption of proteins on the membrane surfaces must be reduced. Natural polymers [8–11] including cellulose and chitosan are resistant to the nonspecific adsorption of most proteins, however, they are biodegradable and thus their in-service time as affinity membranes for bioseparation is relatively limited. Among the synthetic polymers, polyacrylonitrile-based copolymers may be promising candidates for the preparation of affinity membranes, owing to their excellent film- and fiber-forming performance, good mechanical property, and multi-functionality stemming from copolymerization. More interestingly, these copolymers can be facilely fabricated into nanofibrous mats by electrospinning. These nanofibrous mats have much larger specific surface area and higher porosity than traditional membranes [12–14]. The feasibility of nanofibrous mats in affinity separation was first explored by Ma et al. [15]. Cellulose and polysulfone nanofibrous mats were successfully prepared and surface functionalized for protein separation [15–18]. Electrospun affinity membranes for metals separation were also fabricated from chitosan and polyacrylonitrile by Park et al. [19,20]. These nanofibrous affinity mats show strong binding specificity and high capacity to the corresponding targets, which implies they are a kind of novel and promising membranes for protein separation.

Lectins are carbohydrate-binding proteins or glycoproteins that are highly specific for their carbohydrate moieties [21,22]. For examples, lectin concanavalin A (Con A) has affinity interaction with glucose and lectin peanut agglutinin (PNA) with galactose. Therefore, affinity membranes can be expected using specific carbohydrates as ligands to realize the separation of lectins on the basis of carbohydrate–protein interactions [23,24]. GNMs, in which there are carbohydrate ligands decorated on the nanofiber surface, are envisaged as excellent representatives. Two methods have been reported so far to prepare the GNMs as affinity membranes.

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One is to synthesize glycopolymers followed by direct electrospinning [25]. Generally, the synthesis of carbohydrate-containing monomers is relatively complex and most of the carbohydrate ligands are easily entrapped into the glycosylated nanofibers without effective utilization. The other is to surface-modify a nanofibrous mat by the attachment of carbohydrate ligands [26]. This method is more feasible due to the easy accessibility of carbohydrates for protein recognition.

In this paper, we describe a simple approach to prepare nanofibrous affinity membrane decorated with glucose ligands for high specificity and large capacity to lectin Con A. As schematically shown in Fig. 1, poly(acrylonitrile-*co*-hydroxyethyl methacrylate) (PAHM) was synthesized and fabricated into nanofibrous mat by electrospinning. Surface glycosylation was then used to covalently attach glucose ligands on the PAHM nanofibrous mat under a mild condition. Static and dynamic protein adsorption on this GNM was studied comparatively. It selectively recognizes lectin Con A while shows almost no affinity binding with another lectin PNA. We suggest that this GNM is promising in affinity membrane chromatography for lectin separation.

2. Experimental

2.1. Materials

PAHM was synthesized by a water phase precipitation copolymerization method [27]. Its viscosity-average molecular weight was 1.8×10^5 g/mol and the content of 2-hydroxyethyl methacrylate was 11.2 mol%. β-Glucose pentaacetate (GPA) was a product of D-glucose by acetylation. Con A (Vector Laboratories, USA), PNA (Vector Laboratories, USA), bovine serum albumin (BSA, Sino-American Biotechnology Co., China) and D-glucose (Chemical Reagent Co., China) were purchased and used as-received. Boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$, Shanghai Lingfeng Chemical Reagent Co. Ltd., China) and *N*,*N*-dimethylformamide (DMF, Shanghai Chemical Agent Co., China) were commercial products and purified by distillation under reduced pressure before use. Dichloromethane and methanol were analytical grade and dried with 4 Å molecular sieves. 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES, Nanjing Robiot Co. Ltd., China) and sodium methoxide (Sinopharm Group Chemical Reagent Co. Ltd., China) were commercially purchased and used without further purification. All the other chemicals were of analytical grade and used as-received. Water used in the work was de-ionized and ultrafiltrated to 18 MΩ with an ELGA LabWater system.

2.2. Electrospinning of PAHM

Polyacrylonitrile-based nanofibrous mat was prepared by a typical electrospinning process. PAHM powder was dissolved in DMF at room temperature with a gentle stirring for 12 h to form a homogeneous solution of 4 wt.%. After removing the air bubbles completely, the solution was poured into a plastic syringe. The feed rate of spinning solution was controlled to be 1.0 mL/h by a micro-infusion pump (WZ-50C2, Zhejiang University Medical Instrument Co. Ltd., China). A voltage of 12.0 kV/15 cm was applied by a high voltage power supply (GDW-a, Tianjin Dongwen Highvoltage Power Supply Plant, China) with a current output of 0.000–0.001 mA between a stainless steel needle (inner diameter 1.2 mm) at the end of the syringe and a ground collector (aluminum sheet on a flat glass). The process was carried out at ca. 28 °C and in an atmosphere of 40–50% relative humidity. After 5-h deposition, PAHM nanofibrous mat with enough thickness (ca. 15 μm) as membrane was detached from the collector. It was then dried under reduced pressure at 80 °C to remove any residual solvent.

2.3. Surface glycosylation

Surface glycosylation was conducted as reported in our previous work [28]. A sample of PAHM nanofibrous mat was fully immersed in dichloromethane solution (15 mL) in the presence of excess of GPA and boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$, the mole ratio of GPA/$BF_3{\cdot}Et_2O$ is 5:1). The reaction was carried out in nitrogen atmosphere at 0 °C for 2 h followed by 20 h at room temperature. Then, deprotection of the acetyl groups was performed in sodium methoxide/methanol solution (0.1 M, 10 mL) for 90 min at room temperature. The GNM was washed with de-ionized water several times followed by drying under reduced pressure overnight at 60 °C.

Acidic hydrolysis of the GNM was fulfilled at 120 °C for 3 h in trifluoroacetic acid (TFA) solution (3 M). Subsequently, phenol/sulfuric acid assay was employed to measure the amount of free glucose dissociated from the membrane surface [29,30]. Thus, glucose density on the membrane surface was calculated according to the mass ratio of glucose to the GNM.

2.4. Static protein adsorption

Con A solutions with various concentrations were prepared using HEPES buffer solution (pH 8.5, containing 10 mM HEPES and 0.1 mM $CaCl_2$). BSA was used as a reference protein and dissolved in the phosphate buffer solution (PBS, pH 7.0, 10 mM). Glucose solution with a concentration of 1 M in HEPES was employed to elute the adsorbed Con A on the GNM surface. Equal amounts of PAHM nanofibrous membrane and GNM were fully immersed into Con A solutions and/or BSA solutions with different concentrations at 25 °C for 3 h, respectively. The solutions before and after adsorption were detected at 280 nm by UV-vis spectrophotometer (UV-2450, Shimadzu, Japan). Desorption was carried out by immersing the Con A-adsorbed GNM into a glucose solution (1 M) overnight.

2.5. Dynamic protein adsorption

Eight pieces of the GNM (ca. 15 μm per piece) were stacked together and packed into a stainless steel filter holder (Millipore, USA). Protein solution was then pumped into the holder with a peristaltic pump (BT300-1F, Baoding Longer Precision Pump Co.,Ltd., China) at a flow rate of 248.6 μL/min (the same below). The effluent was collected and detected every 3 min by UV-vis spectrophotometer at 280 nm. After protein adsorption, HEPES buffer solution was pumped into the filter system until no protein was detected in the effluent. Then, the glucose solution (1 M) was injected to elute the adsorbed protein from the GNM. Finally, the filter system was washed by HEPES buffer solution again and the membrane was extracted and conserved in the buffer.

2.6. Characterization

Field-emission scanning electron microscopy (FESEM, SIRION-100, FEI, Netherlands) was used to characterized the morphologies of the nanofibrous membrane before and after glycosylation. X-ray photoelectron spectroscopy (XPS) measurements were carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer, USA) with Mg Kα radiation ($h\nu$ = 1253.6 eV). Binding energies were calibrated with the containment carbon (C 1s = 284.6 eV).

3. Results and discussion

3.1. Preparation and characterization of the GNM

Polyacrylonitrile (PAN)-based copolymers with different functional groups have been designed and synthesized in our laboratory