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Immunoreaction-based separation of antibodies using gold nanotubules membrane

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Abstract

A new method for protein separation by the gold nanotubules membrane based on the immunoreaction between antibody and antigen was described. The gold nanotubules can be electrolessly plated within the pores of polycarbonate nanoporous filtration membranes. The capture antibody, bovine IgG, was modified onto the inner wall of Au nanotubules by glutaraldehyde interlinking method. The transport behaviour of detecting antibody through the nanotubules modified with capture antibody was investigated. When the diameter of the nanotubule was much larger than the size of the antibodies, there was no obvious difference between the transport rates for the proteins studied here through nanotubules. When the diameter of the nanotubule was ca. 30 nm, the transport of the goat anti-cat antibody through the nanotubules membrane was slow. Compared with goat anti-cat IgG, goat anti-bovine antibody transported through nanotubules membrane faster due to the immunoreaction between goat anti-bovine antibody and bovine IgG modified onto the pores of the membrane and the separation efficiency was 5.6. The results indicated that the gold nanotubule membranes showed good separating capability for protein without the loss of activity of the antibodies.

Keywords: Nanotubule membrane; Antibody; Transporting; Separation; Self-assembly

1. Introduction

Separation and purification of proteins is very important in the biochemical engineering, protein analysis, medical inspection of drugs. Many methods, such as deposition method [1], adsorption chromatography [2], hydrophobic chromatography [3], ion exchange chromatography [4], gel filtration method [5] and affinity chromatography [6], etc., have been used for the separation of proteins based on the size, shape, adsorption property and acidity or alkalescence of substances. However, several significant challenges still exist in this field. For example, it is difficult to control the condition of separation of these methods [7,8], and the selectivity of these methods is not so good. Some new separation techniques such as transfer medium column [9] have been studied for the separation of protein. Although these techniques offered the advantage of short transfer distance of substance passed through integration micro-pores, the recovery of separated protein is lower.

Recently, the effective separation with high recovery and analysis of protein has received great attention in the chemical and biological field. Many compounds have been separated by the Au nanotubules based on the sizes of molecules and/or ions and charge of the compounds [10–15]. Chemical sorption of thiols onto the Au nanotubules enhanced transport selectivity [16,17]. Martin and his coauthors [18] have reported the size-based protein separations with the poly(ethylene glycol)-derivatized gold nanotubule membranes. Chun and Stroeve [19] separated BSA and bovine hemoglobin (BHb) using Au-Mem modified with alkane thiol ($HSC_{10}H_{20}COOH$) with a diameter of 8.7 nm. Their research results showed that for the mixed protein separation, the highest selectivity was 4.3. Huang et al. [20] investigated the transport property of BSA through the nanomembrane modified with cystein. The results showed that the modifier facilitated transporting of BSA through the nanotubules membrane. As far as our knowledge goes, there are few reports on the transporting of protein based on the immunoreaction by the Au nanotubules.

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IgG molecules are potential neuropharmaceuticals that may be used for therapeutic or diagnostic purposes. Goat anti-bovine (IgG G-anti-B-IgG) and goat anti-cat IgG (G-anti-C-IgG) have nearly identical molecular structure and size. However, the membrane separation of similar size proteins is difficult [19]. In the present work, we have been exploring the transport and separation of G-anti-B-IgG and G-anti-C-IgG through Au nanotubules, polycarbonate filtration membrane based on the immunoreaction between antigen and antibody. When the diameter of the nanotubule was much larger than the size of the antibodies, the difference of fluxes of the proteins through the nanotubules membrane was not obvious. However, the flux of the proteins through membrane was increased with reducing the diameter of the nanotubules. The modification of bovine IgG has a great effect on the transport of G-anti-B-IgG and G-anti-C-IgG through the Au nanotubules. We demonstrated that the immunoreaction between G-anti-B-IgG and bovine IgG is an important factor in separation of G-anti-B-IgG and G-anti-C-IgG based on Au nanotubules membrane. The method proposed here solved the problem of adsorption during separation process, resulting in an improving of separation efficiency. The method provides a convenient, rapid and sensitive separation way for antibodies.

2. Experimental

2.1. Reagents and apparatus

Polycarbonate nanoporous filtration membranes with pore diameters of 100 nm, thickness of 6 μm, and pore densities of 6×10^8 pores/cm^2 (Millipore company) were used to prepare Au nanotubules. Bovine IgG, goat anti-bovine IgG(G-anti-B-IgG) labeled with Texas red (TXRD-G-anti-B-IgG) and goat anti-cat IgG antibodies(G-anti-C-IgG) labeled with isothiocyanate (FITC-G-anti-C-IgG) were obtained from Beijing DingGuo Biotechnology Center. Milli Q 18.3 MΩ water was used throughout the experiments. Other reagents were of analytical reagent grade.

A SPA-400 atomic force microscope (AFM) (Seiko, Japan) and scanning electron microscope (SEM) were used to characterize the Au nanotubules. F-2500 fluorescence spectrophotometer (Hitachi, Japan) was used to measure the fluorescence intensity.

The schematic drawing of separation cell based on nanotubules was the same as that described in the report [21]. Before every experiment, solutions containing antibody to be tested were put into the feed cells while solvents of the same volume as to-be-tested solutions were put into permeate cell to keep same height of liquid surface at both cells. Both cells should be sampled, respectively, at the same time for measurements of fluorescence intensity. In other words, the equal liquid level between feed cell and permeate cell was maintained during the experiment. After each measurement, the test solutions were injected into transport cells back to keep comparability of the results. All transport measurements were performed in 0.01 M NaCl.

2.2. Procedure

2.2.1. Preparation of Au nanotubules

Au nanotubules was prepared according to a modified method proposed by Martin and coworkers [10,16]. The polycarbonate nanoporous filtration membrane was immersed into methanol for 5 min and then immersed in a solution of 0.025 M in $SnCl_2$ and 0.07 M in trifluoroacetic acid for 45 min. The membrane was placed in methanol two consecutive times for 2.5 min each and then immersed in an aqueous ammoniacal $AgNO_3$ solution (0.029 M) for 5 min, followed by soaking it in methanol for 5 min. After treatment in $AgNO_3$, the membrane was placed in the gold-plating bath containing 0.127 M Na_2SO_3, 0.625 M formaldehyde and 0.025 M $NaHCO_3$. The temperature of this bath was maintained at 5 °C. The plating solution was adjusted to pH 10.87 by dropwise addition of 0.5 M H_2SO_4, with constant stirring. The Au nanotubule membrane was immersed in 25% (v/v) nitric acid to remove impurities absorbed on the surface of the membrane, followed by rinsing with water twice.

2.2.2. Immobilization of capture antibody

A covalent cross-linking method was used for the immobilization of capture antibody onto the inner wall of the nanotubules. Immobilization of bovine IgG was carried on according to the following procedures:

- *Activation of the membrane*. The nanotubules membrane was washed with a Piranha solution (H_2SO_4:H_2O_2 = 7:3, v/v), then rinsed successively with alcohol and water thoroughly.
- *Modification of Au nanotubules*. The gold nanotubules membrane was immersed in a solution of cystine for 12 h at room temperature in a nitrogen atmosphere, then rinsed with PBS buffer solution to remove the unnecessary cystine, followed by soaking in a solution of 2.5% in glutaraldehyde for 4 h. The resulting membrane was soaked in the solution of 100 μg/mL in bovine-IgG for 24 h at 4 °C, then rinsed with PBS buffer solution.

2.2.3. Obturation of Au nanotubules

The membrane modified with bovine IgG was soaked in a 0.2 M L-lysine solution to obturate excrescent aldehyde groups of glutaric dialdehyde, followed by rinsing with the PBS buffer solution thoroughly. The Au nanotubules membrane modified with bovine IgG was soaked in the PBS solution at 4 °C before use.

In this paper, PC-Mem, Au-Mem and B-IgG-Au-Mem were used to denote the naked polycarbonate nanoporous filtration membrane, polycarbonate nanoporous filtration membrane covered with gold, Au-nanoporous filtration membrane modified with bovine-IgG, respectively.

2.2.4. Determination of TXRD-goat anti-bovine IgG and FITC-goat anti-cat IgG

The permeation content of TXRD-goat anti-bovine IgG and FITC-goat anti-cat IgG through the nanotubules membrane was monitored by measuring the fluorescence intensity of the detecting antibodies in permeate cell as a function