

Note

INTERACTION BETWEEN THE SURFACE GLYCOSYLATED POLYPROPYLENE MEMBRANE AND LECTIN*

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Abstract A glycopolymer bearing glucose residues was tethered onto the surface of polypropylene microporous membrane by UV-induced graft polymerization of α -allyl glucoside. Concanavalin A (Con A), a glucose recognizing lectin, could be specifically adsorbed to the membrane surface. On the other hand, the membrane surface showed no recognition ability to another lectin peanut agglutinin. Moreover, the recognition complex between the glycosylated membrane surface and Con A could be inhibited by glucose and mannose solution. This surface glycosylated membrane could be used as affinity membrane for protein separation and purification.

Keywords: Affinity membrane; Protein separation; Glycopolymer; Glycosylated surface; Graft polymerization; Polypropylene microporous membrane.

INTRODUCTION

Protein separation is very important in modern industry, especially in biotechnological and pharmaceutical ones. Conventional column chromatography has been widely used for protein separation^[1]. However, this technique suffers diffusion limitation and is not cost effective^[2–5]. Therefore, there is a great need to develop an affinity membrane to overcome the problems of limited specificity experienced with membranes that operate purely on a sieving mechanism^[3] and capable of the selective capture of one specific protein.

Generally, the affinity membrane process has three steps^[6]: (1) immobilization of a ligand that has specific affinity towards the target protein on the membrane, (2) selective attachment of the target protein from a mixture to the membrane by ligand-protein interaction, (3) purification of the attached protein and elution from the membrane by disrupting ligand-protein interaction. Thus, the interactions used in affinity membrane should be highly selective, enough strong and reversible. This selectivity is based on specific ligand-protein interactions. The commonly adopted interactions are avidin-biotin, antigen-antibody, enzyme-substrate, or oligonucleotide-protein binding^[3]. Though the specificity and the strength are very high, there are few work^[7] based on carbohydrate-protein interaction. Carbohydrate-protein interaction is one of the most important interactions in the biologic system. This interaction is involved in many vital biological processes such as blood coagulation, immune response, viral infection, inflammation, embryogenesis and cellular signal transfer^[8–10]. Almost all the biological functions of carbohydrates are achieved by the specific recognition against proteins which relies on the aggregation of the carbohydrate ligands^[11, 12]. Recently, a large number of different synthetic multivalent glycoligands, among which glycopolymer may be the most attractive one, have been designed to improve the

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strength of binding and also its specificity. On the other hand, the carbohydrate-protein interaction is reversible which can be inhibited by corresponding monosaccharides^[13]. Take the advantages of high specificity, strong strength and reversibility, the interaction between carbohydrate ligands and proteins can be endowed to high effective affinity membrane. In our previous work^[14–16], glycopolymers bearing pendant glucose were grafted onto polypropylene microporous membrane (PPMM) to give high valency ligand tethered surfaces. The main objective of this research was to evaluate the specific interaction between the glycosylated membrane surface and protein by recognition and inhibition experiments. One can envisage that, combined with the separation ability, this surface glycosylated membrane is very promising in protein separation and specific capture of virus.

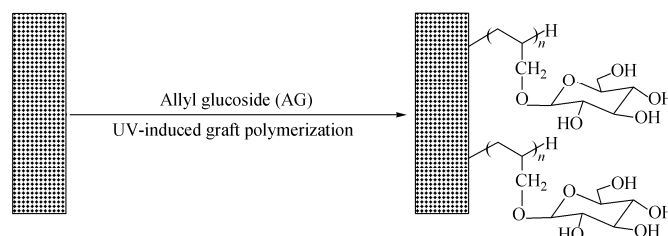
EXPERIMENTAL

Materials and Chemicals

PPMM was purchased from Membrana GmbH (Germany), which was prepared by thermally induced phase separation (TIPS) method with an average pore size of 0.20 μm and porosity about 75%. HEPES, fluorescein-labeled peanut agglutinin (FL-PNA, Vector), and fluorescein-labeled concanavalin A (FL-Con A, Vector) were used as received. The water used in all synthesis and measurements was de-ionized and ultrafiltrated to 18 M Ω .

Surface Glycosylation of PPMM

Poly(α -allyl glucoside) (PAG) grafted PPMM (PPMM-*g*-PAG, Scheme 1) was prepared by UV-induced graft polymerization as described previously^[14]. Briefly, PPMM was dipped in 10 mL photo-initiator solution (benzophenone in heptane) for 60 min, and then dried in air for 30 min. Thereafter, the membrane was washed with acetone and quickly wiped with filter paper. The membrane with pore still wetted by acetone was fixed between two filter papers (No. 593, Schleicher & Schuell) immediately and immersed into 10 mL monomer solution in a reaction chamber. Then, UV irradiation was done for a predetermined time under argon gas environment in an ultraviolet processor. The processor was equipped with two 300 W high pressure mercury lamp. The distance between the lamp and the reaction chamber was 25 cm. Finally, the membrane was washed with acetone and water drastically using a vibrator.



Scheme 1 Schematic diagram illustrating the UV-induced graft polymerization of AG on PPMM

Lectin Recognition on PPMM-*g*-PAG Surface

PPMM-*g*-PAG sample with diameter of 5 mm was immersed in 200 μL FL-Con A or FL-PNA solution (0.2 mg/mL in 10 mmol/L HEPES, pH = 7.5, containing 0.15 mol/L NaCl, 0.1 mmol/L Ca^{2+} and 0.01 mmol/L Mn^{2+}) and incubated at 25°C for 2 h. After washed with HEPES solution, the fluorescence images were observed with a fluorescence microscope (Olympus IX71, Japan).

Inhibition of Surface-Recognized FL-Con A by Monosaccharides

FL-Con A recognized PPMM-*g*-PAG sample was immersed in 200 μL monosaccharides solution (glucose, mannose and galactose respectively in HEPES) and incubated at 25°C for 24 h. Then, the sample was rinsed six times with 200 μL HEPES each. The inhibition solution and the eluent were collected together and diluted to 3 mL with HEPES. Shimadzu RF-3510PC fluorescence spectrophotometer and matched quartz cells of 1 cm path length were used to obtain the fluorescence spectrum.

RESULTS AND DISCUSSION

Lectin Recognition on the PPMM-g-PAG Surface

Con A is a lectin which can specifically recognized by glucose and mannose. From the fluorescence images in Fig. 1 we can clearly see the bonding of FL-Con A to the PPMM-g-PAG surface. The nascent PPMM showed very weak fluorescence which was caused by physical adsorption of FL-Con A. The PPMM-g-PAG surface with low grafting density also exhibited very weak interactions with FL-Con A and the fluorescence intensity was almost the same as that of the nascent PPMM. When the sugar density on the membrane surface increased

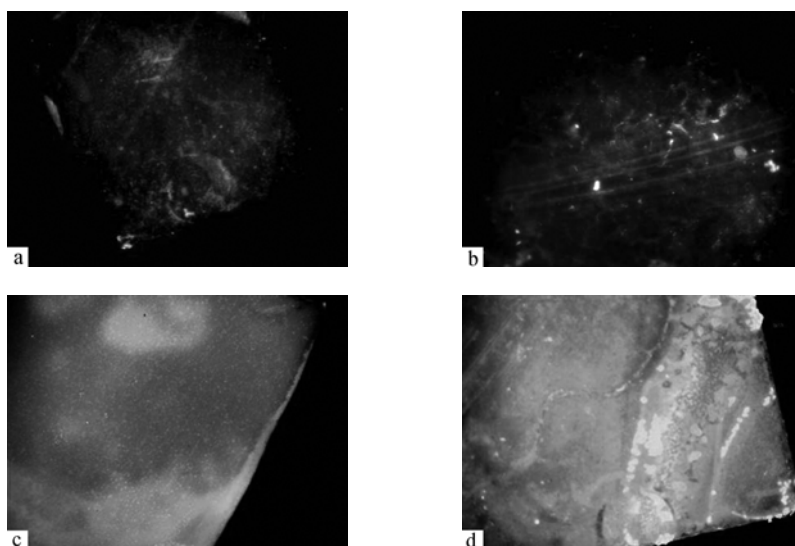


Fig. 1 Fluorescence microscope images of PPMMs after FL-Con A adsorption: (a) nascent, (b–d) PPMM-g-PAG with grafting densities of 89.80, 187.76 and 220.41 $\mu\text{g}/\text{cm}^2$ respectively. All the images were taken at 40 $\times$ magnification.

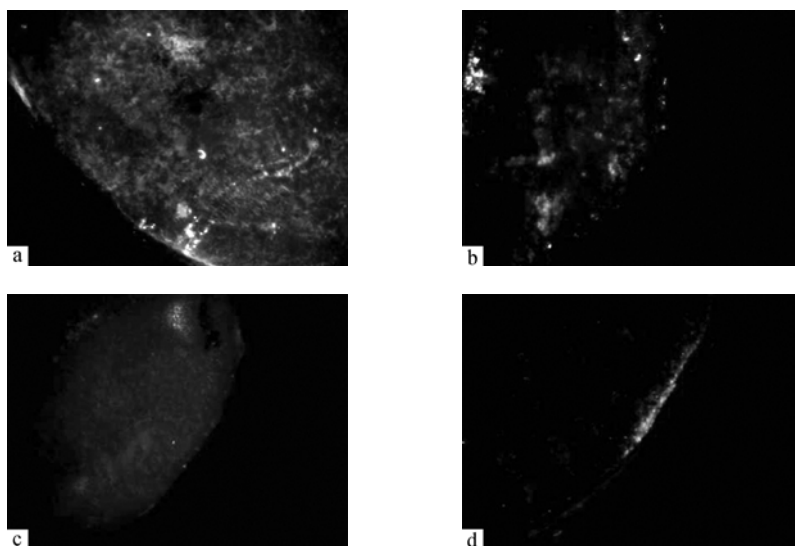


Fig. 2 Fluorescence microscope images of PPMMs after FL-PNA adsorption: (a) nascent, (b–d) PPMM-g-PAG with grafting densities of 89.80, 187.76 and 220.41 $\mu\text{g}/\text{cm}^2$ respectively. All the images were taken at 40 $\times$ magnification.

to $220.41 \mu\text{g}/\text{cm}^2$, the interaction between FL-Con A and glucose residues on the membrane surface increased obviously as a result of the “glycoside cluster effect”, therefore, FL-Con A adsorbed to the membrane surface dramatically and bright fluorescence could be observed. On the contrary, PNA is a lectin binding to galactose residue and shows no specific interaction with glucose. As can be seen from Fig. 2, no obvious fluorescence was detected from the studied membrane surface after FL-PNA adsorption. This result indicates that almost no FL-PNA was recognized to the membrane surface. Low fluorescence in the images was also caused by physical adsorption.

Inhibition of Surface-Recognized FL-Con A by Monosaccharides

The complex between lectins and glycoligands can be inhibited by the sugar ligand which the lectins are specific^[13]. Figure 3 shows the fluorescence images of FL-Con A recognized PPMM surfaces after inhibiting with 0.5 g/L glucose solution. As can be seen, FL-Con A banded on the PPMM-*g*-PAG surfaces with higher sugar densities (Figs. 3c and 3d) was inhibited by glucose solution and the fluorescence intensity decreased obviously. At the same time, we can observe that the PPMM-*g*-PAG surface with sugar density of $220.41 \mu\text{g}/\text{cm}^2$ showed considerable fluorescence after inhibition with 0.5 g/L glucose solution. This can be ascribed to the strong interaction between the high sugar density surface and FL-Con A. With different sugar concentration, a series inhibition effects could be achieved. As showed in Figs. 4(a) and 4(b), glucose and mannose exhibit similar inhibition effect. With increase of the sugar concentration, the fluorescence intensity of the eluent increased first and then reached a platform which indicating the complete inhibition of the recognized Con A. On the other hand, galactose, which is not a Con A binding sugar, has no inhibition effect on the membrane surface recognized Con A and the eluent solution showed almost no fluorescence.

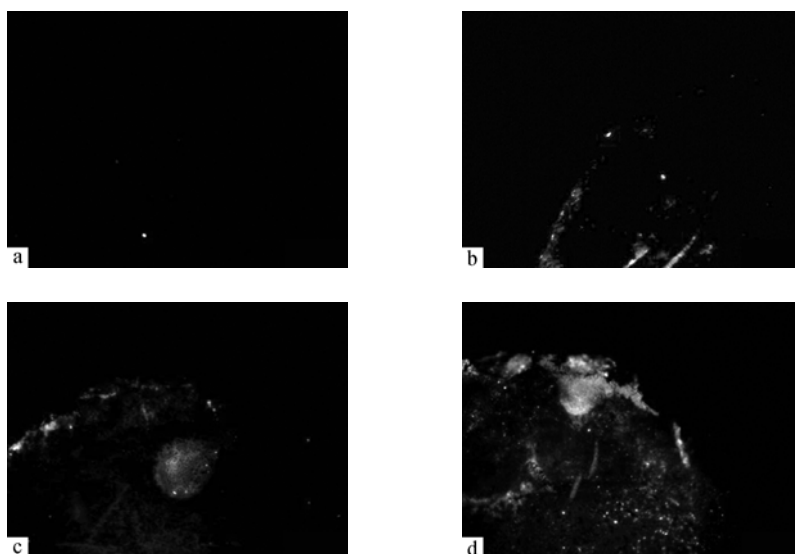


Fig. 3 Fluorescence microscope images of FL-Con A adsorbed PPMMs after inhibiting with 0.5 g/L glucose solution: (a) nascent, (b–d) PPMM-*g*-PAG with grafting densities of 89.80, 187.76 and $220.41 \mu\text{g}/\text{cm}^2$ respectively

All the images were taken at $40\times$ magnification.

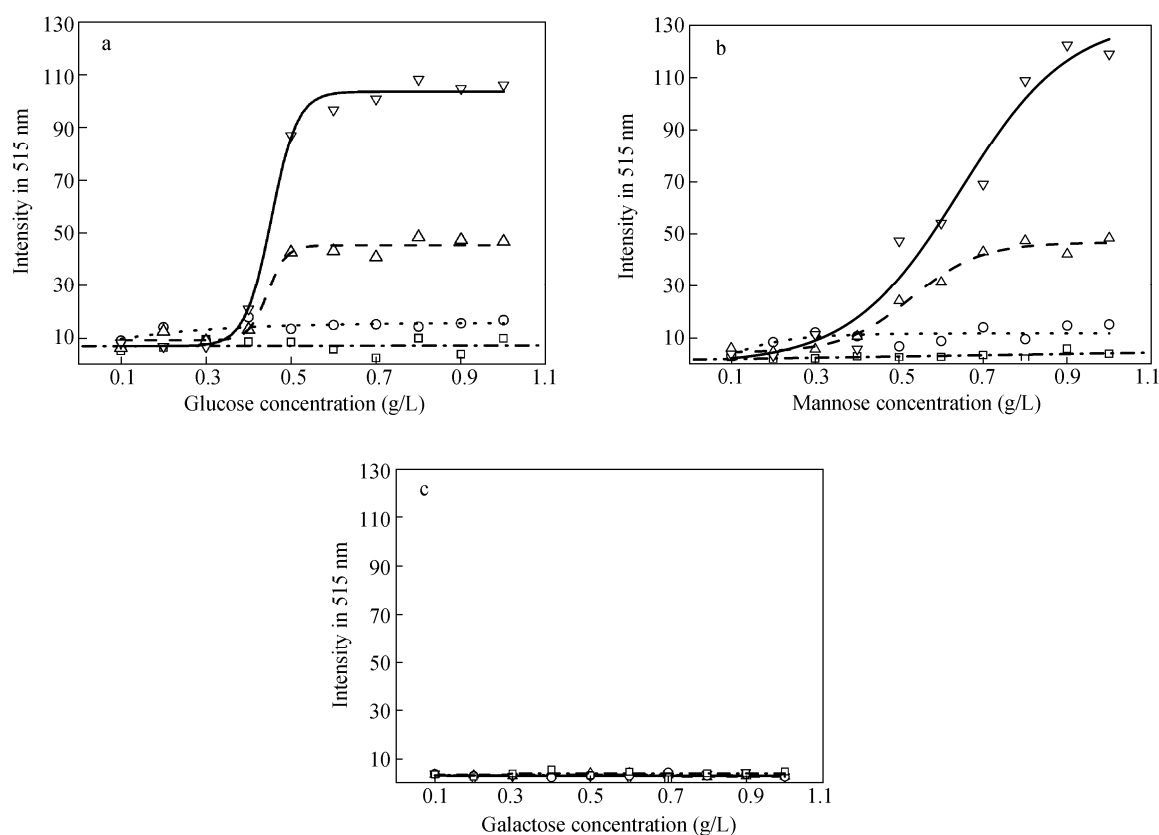


Fig. 4 Effect of glucose, mannose and galactose solution concentrations on the fluorescence intensity of the eluent in 515 nm
 PAG grafting densities on PPMM: 220.41 (∇), 187.76 ($\triangle$), 89.80 ($\circ$) and 0 ($\square$) $\mu\text{g}/\text{cm}^2$ respectively

CONCLUSIONS

PPMM was glycosylated by tethering glycopolymers onto the surface. FL-Con A and FL-PNA were used to evaluate the specificity of the interaction between the glycosylated membrane surface and the proteins. Inhibition experiment revealed that the recognition complex could be inhibited by glucose and mannose solutions and the specifically recognized protein could be recovered from the membrane surface.

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