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Fabrication of Glycosylated Surface on Polymer Membrane by UV-Induced Graft Polymerization for Lectin Recognition

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Increasingly, carbohydrate−protein interactions are viewed as important mechanisms for many biological processes such as blood coagulation, immune response, viral infection, inflammation, embryogenesis, and cellular signal transfer. However, the weak affinity of the interactions and the structural complexity of carbohydrates have hindered efforts to develop a comprehensive understanding of carbohydrate functions. Fortunately, synthetic polyvalent glycoligands give us a chance to reveal the nature of these biological processes. In this work a sugar-containing monomer (α-D-allyl glucoside (AG)) was grafted onto polypropylene microporous membrane (PPMM) by UV-induced graft polymerization to generate a glycosylated porous surface for the first time. Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, and scanning electron microscopy were employed to confirm the glycosylation. Water contact angle measurement was used to evaluate the hydrophilicity change of the surfaces before and after the graft polymerization of AG. It was found that the grafting density increased reasonably with the increase of AG monomer concentration, and then this increase slowed when the AG concentration exceeded 80 g/L. At the same time a 20−25 min UV irradiation was enough for the grafting polymerization. The photoinitiator concentration also influenced the grafting density obviously, and there was an optimal concentration of the photoinitiator for the grafting process. The water contact angle of the polyAG-tethered membrane surface decreased from 149° to 80° with the increase of grafting density from 0 to 187.76 μg/cm^2, which indicated a hydrophilic variation of the membrane surface by the grafting of AG. Results also indicated that the surface-grafted polyAG chains showed weak interaction with Con A when the grafting density was low. However, when the sugar density exceeded 90 μg/cm^2, the binding affinity increased dramatically which was the due to the "glycoside cluster effect".

Introduction

Glycobiology deals with the role of carbohydrates in biological events and has become a popular topic in recent years because of the number of structurally and functionally diverse roles that the carbohydrates display, e.g., as energy sources and elements for recognition with proteins.[1] Though the precise mechanism and exact structures of the carbohydrates in the carbohydrate−protein interactions is still not well known, this recognition is widely accepted to be the key in a variety of biological processes and the first step in numerous phenomena based on cell−cell interactions, such as blood coagulation, immune response, viral infection, inflammation, embryogenesis, and cellular signal transfer.[2−4] For example, heparin, a sulfated polysaccharide composed mostly of glucuronic acid and glucosamine, plays an important role in the blood coagulation system.[5] Carbohydrates have been found on the surface of nearly every cell in the form of polysaccharides, glycoproteins, glycolipids, and/or other glycoconjugates. They serve as sites for docking other cells, molecules, and pathogens in a more or less specific recognition process, which is eventually triggered by these molecular interactions.[6]

Despite the essentiality and importance of carbohydrates in biological processes, the difficulty in studying the carbohydrate−protein interactions has hindered efforts to give a perspicuous comprehension.[7] A major difficulty stems from the weak binding affinities which range between 10^3 and 10^4 M^{-1}.[8] Indeed, in nature, carbohydrate-binding proteins are typically aggregated into higher order oligomeric structures, which suggests that the binding limitations can be circumvented through multivalency. The strength of binding and also its specificity can be improved by multivalent interactions, which have been found quite regularly in biosystems. The mechanism by which multivalent ligands acted is still not very well known, but it is increasingly accepted that the "cluster glycoside effect" relies on aggregation.[9] Therefore, understanding the molecular principles underlying multivalency effects is an important goal of biological chemistry. Consequently, a large number of different synthetic multivalent glycoligands (glycoclusters, glycodendrimers, and glycopolymers etc.) have been designed to interfere effectively with carbohydrate−protein interactions and facilitate investigation of the multiple interactions occurring during these molecular recognition events.[10−17] Among these manmade multivalent carbohydrate

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(1) Varki, A. *Glycobiology* **1993**, *3*, 97−130.
(2) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491−514.
(3) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431−449.
(4) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720.
(5) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327.
(6) Lindhorst, T. K. *Curr. Top. Chem.* **2002**, *218*, 201−235.
(7) Houseman, B. T.; Mrksich, M. *Curr. Top. Chem.* **2002**, *218*, 1−44.
(8) Nagahori, N.; Nishimura, S. *Biomacromolecules* **2001**, *2*, 22−24.
(9) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578.
(10) Benito, J. M.; Gomez-Garcia, M.; Mellet, C. O.; Baussanne, I.; Defaye, J.; Fernandez, J. M. G. *J. Am. Chem. Soc.* **2004**, *126*, 10355−10363.
(11) Wolfenden, M. L.; Cloninger, M. J. *J. Am. Chem. Soc.* **2005**, *127*, 12168−12169.
(12) Bandaru, N. M.; Sampath, S.; Jayaraman, N. *Langmuir* **2005**, *21*, 9591−9596.
(13) Serizawa, T.; Yasunaga, S.; Akashi, M. *Biomacromolecules* **2001**, *2*, 469−475.
(14) Takasu, A.; Iso, K.; Dohmae, T.; Hirabayashi, T. *Biomacromolecules* **2006**, *7*, 411−414.
(15) Hasegawa, T.; Kondoh, S.; Matsuura, K.; Kobayashi, K. *Macromolecules* **1999**, *32*, 6595−6603.
(16) Muthukrishnan, S.; Jutz, G.; Andre, X.; Mori, H.; Muller, A. H. E. *Macromolecules* **2005**, *38*, 9−18.

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ligands, glycopolymers continue to be attractive because of the very high valency, it is easy to control the molecular structure, and it is facile to vary the species of the sugar ligands.[18−20] However, bulk glycopolymers often have the disadvantage of great steric hindrance. The intertwists of the polymer chains result in low effective sugar residues for recognition of proteins. Therefore, grafting glycopolymer chains directly to solid supports (flat or spherical) becomes attractive because the tethered polymer brushes are usually stretched away from the substrates to avoid overlapping. Herein, we report an effective way to generate glycopolymer brushes on porous support, polypropylene microporous membrane (PPMM), which brings relatively high valency and control over sugar density. The porous structure offers a relatively large surface area for tethering of glycopolymer chains, and subsequently, high sugar density can be achieved. UV-induced graft polymerization was employed to tether the glycopolymer to the support surface, and the grafting process was investigated. Concanavalin A, one kind of lectin, was used as model protein to evaluate the recognition property of this surface-immobilized polyvalent ligands. Combined with the separation ability, this surface glycosylated membrane is very promising in protein separation and drug delivery systems. Moreover, the described glycosylation strategy can also be extended to other polymer materials such as nanoparticles and nanofibers, which are prospects for obtaining high-valency glycoligands.

Experimental Section

Materials. Polypropylene microporous membrane (PPMM) was purchased from Membrana GmbH (Germany), which was prepared by a thermally induced phase separation (TIPS) method with an average pore size of 0.20 μm and a relatively high porosity of about 80%. All membranes used in this study were cut into a round shape with a diameter of 3.95 cm (area = 12.25 cm^2). Before graft polymerization, the membranes were dipped in acetone for 30 min and then rinsed with acetone several times to remove any impurities adsorbed on the surfaces. After being dried in a vacuum oven at 40 °C for 1 h, these membranes were stored in a desiccator. Benzophenone (BP), heptane, $MnCl_2$, $CaCl_2$, and NaCl were analytical grade and used without further purification. Concanavalin A (Con A) was a commercial product in no salt powder form. The water used in all syntheses and measurements was deionized and ultrafiltrated to 18 MΩ.

UV-Induced Graft Polymerization of α-D-Allyl Glucoside. α-D-Allyl glucoside (AG) was synthesized as described previously.[21] AG was dissolved in water to prepare a series of solutions with different monomer concentration. PPMM was dipped in 10 mL of photoinitiator 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 the pore still wetted by acetone was fixed between two filter papers (No. 593, Schleicher & Schuell) immediately and immersed into 10 mL of monomer solution in a reaction chamber. Then, UV irradiation was done for a predetermined time under an argon gas environment in an ultraviolet processor. The processor was equipped with two 300 W high-pressure mercury lamps. 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. After drying in a vacuum oven at 40 °C to constant weight, the grafting density (GD, $\mu g/cm^2$), was calculated by the following equation

$$GD = \frac{W_1 - W_0}{A_m}$$

where W_0 is the mass of the nascent membrane and W_1 is the mass of the membrane after graft polymerization and drying. A_m represents the area of the membrane (12.25 cm^2). Each result was the average of three parallel experiments.

Surface Characterization. To investigate the changes in the surface chemical structure and morphology before and after modification and confirm the grafting and glycopolymer formation, surface characterization techniques were used (attenuated total reflectance Fourier transform infrared spectroscopy (FT-IR/ATR), X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM)).

FT-IR/ATR measurement was carried out on a Vector 22 FT-IR (Brucker Optics, Switzerland) equipped with ATR cell (KRS-5 crystal, 45°). Sixteen scans were taken for each spectrum at a resolution of 4 cm^{-1}. XPS measurements of the original and modified membranes were performed on a PHI-5000C ESCA system (Perkin-Elmer) with Al Kα radiation (h_n = 1486.6 eV). In general, the X-ray anode was run at 250 W and the high voltage was kept at 14.0 kV with a detection angle of 45°. The pass energy was fixed at 93.9 eV to ensure sufficient sensitivity. The base pressure of the analyzer chamber was about 5×10^{-7} Pa. The survey spectra (0−1200 eV) and the core-lever spectra with much high resolution were both recorded. Binding energies were calibrated using the containment carbon (C_{1S} = 284.6 eV). Data analysis was carried out on the PHI-MATLAB software provided by PHI Corp. No radiation damage was observed during the data collection time. SEM images were taken on a field emission SEM (SIRION, FEI).

Water Contact Angle Measurement. An OCA20 contact angle system (Dataphysics, Germany) was used for determination of air/water contact angles at room temperature. Static contact angle was measured by sessile drop method as follows. First, a water drop (∼5 μL) was lowered onto the membrane surface from a needle tip. Then, images of the droplet were recorded. Contact angles were calculated from these images with software. All results were an average of at least five measurements.

Lectin Recognition. Con A (1 g) was dissolved in phosphate-buffered saline solution (PBS, 0.1 M, pH 7.36) containing 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$, and 0.1 M NaCl, and the solution was diluted accurately to 1 L. Membranes with an external surface area of 24.5 (12.25×2) cm^{-2} were cut into pieces about 5×5 mm and immersed in ethanol for 10 min and then in PBS for 30 min to prewet. Then each sample was put into a tube containing 10 mL of Con A solution, and the mixture was incubated at 25 °C for 30 min. The amount of adsorbed Con A was determined by measuring spectrophotometrically the difference between the concentration of Con A in the solution before and after recognition with the sugar residues on the membranes. The spectroscopic analytical method utilized in this work for protein concentration was based on reaction of protein with Coomassie brilliant blue (Fluka) dyestuff to record the absorbance of the protein−Coomassie brilliant blue complex according to Bradford's method.[22] A calibration curve between the spectrophotometric absorbance and the Con A concentration was established to reduce the effect of protein adsorption on the surface of the experimental device for the adsorption measurement.

Results and Discussion

Graft Polymerization. The whole process for the graft polymerization is depicted in Scheme 1. The effect of AG concentration on the GD is shown in Figure 1. As can be seen, AG concentration has a great influence on GD. GD increased with the increase of AG concentration, and the increase exhibited a linear curve at lower concentrations. This could be ascribed

(17) Muthukrishnan, S.; Mori, H.; Muller, A. H. E. *Macromolecules* **2005**, *38*, 3108−3119.
(18) Okada, M. *Prog. Polym. Sci.* **2001**, *26*, 67−104.
(19) Wang, Q.; Dordick, J. S.; Linhardt, R. J. *Chem. Mater.* **2002**, *14*, 3232−3244.
(20) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431−449.
(21) (a) Xu, Z.-K.; Kou, R.-Q.; Liu, Z.-M.; Nie, F.-Q.; Xu, Y.-Y. *Macromolecules* **2003**, *36*, 2441−2447. (b) Xu, Z.-K.; Yang, Q.; Kou, R.-Q.; Wu, J.; Wang, J.-Q. *J. Membr. Sci.* **2004**, *243* (1−2), 195−202.
(22) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

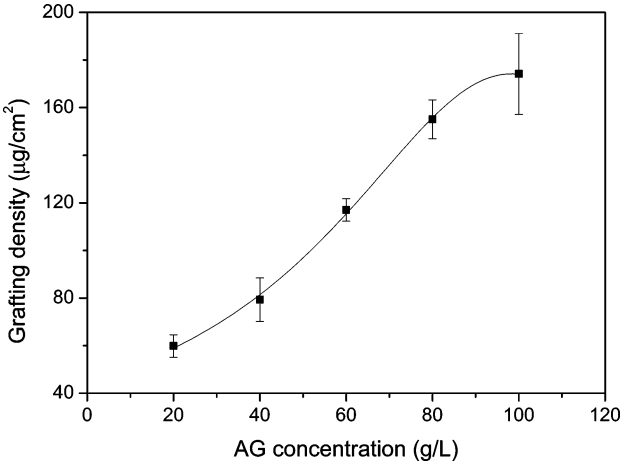


Figure 1. Effect of AG concentration on the grafting density (BP concentration = 3.64 g/L, UV irradiation time = 25 min).

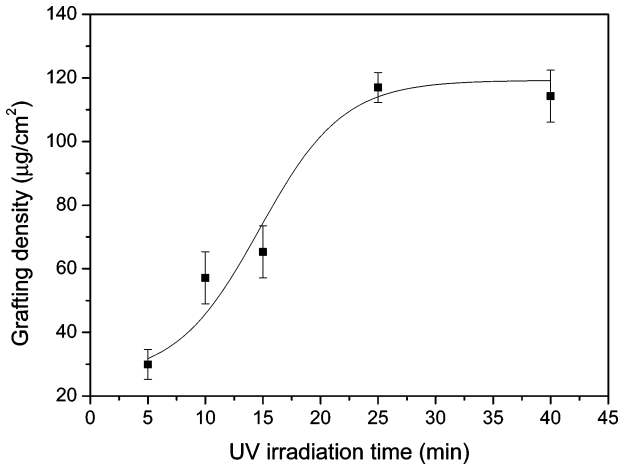
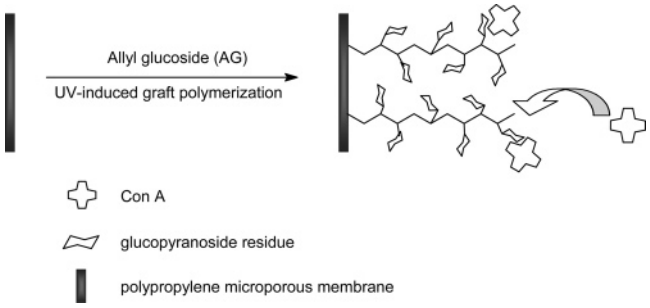


Figure 2. Plot of the grafting density versus UV irradiation time (AG concentration = 60 g/L, BP concentration = 3.64 g/L).

Scheme 1. Schematic Diagram Illustrating the UV-Induced Graft Polymerization of AG



to the increase of monomer concentration; the active sites on the membrane surface had more chances to react with AG monomer and led to higher grafting degree subsequently.However, the tendency to increase slowed when the monomer concentration exceeded 80 g/L. Within the same UV irradiation time (25 min), approximately the same amount of active sites could be generated on the surface and the grafting was not limitless. Also, the viscosity of the monomer solution increased with monomer concentration. The solution became viscous because of the intermolecular hydrogen-bond interactions between glycomonomers, especially in high monomer concentration cases, and these interactions cumbered diffusion of the glycomonomer molecules from the solution to the membrane surface. Moreover, higher monomer concentration may increase the likelihood of producing polymer

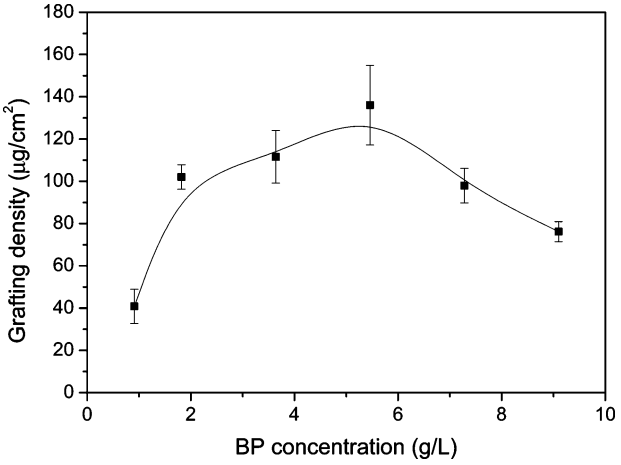


Figure 3. Dependence of the grafting density on BP concentration (AG concentration = 60 g/L, UV irradiation time = 25 min).

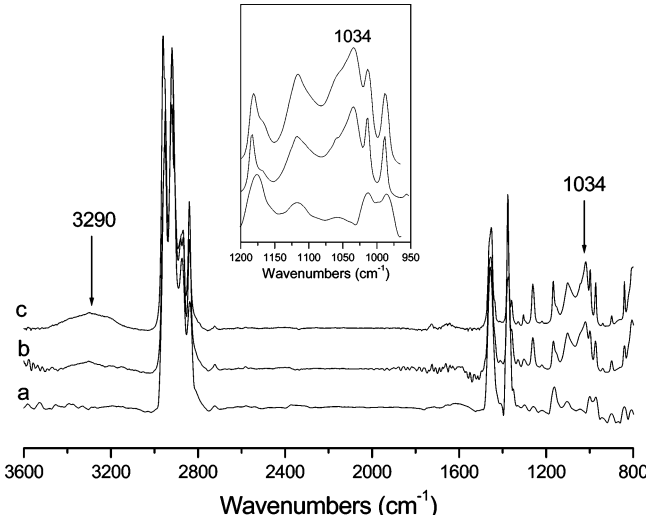


Figure 4. IR spectra of the PPMMs: (a) nascent PPMM, (b) 74.70 μg/cm^2 AG-grafted PPMM, and (c) 122.45 μg/cm^2 AG-grafted PPMM.

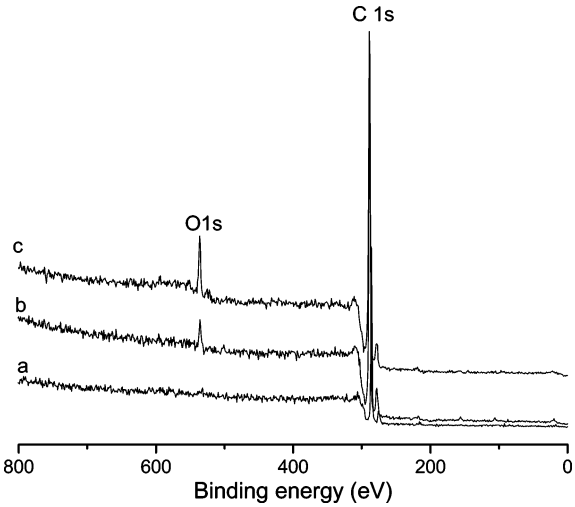


Figure 5. XPS spectra of the nascent and modified PPMMs: (a) nascent PPMM, (b) 65.31 μg/cm^2 AG-grafted PPMM, and (c) 146.94 μg/cm^2 AG-grafted PPMM.

in solution, subtracting monomer to the grafting process. Thus, slowing down of GD occurred at higher monomer concentrations.

Figure 2 shows the effect of the UV irradiation time on the GD. In this experiment 60 g/L AG concentration was used. It

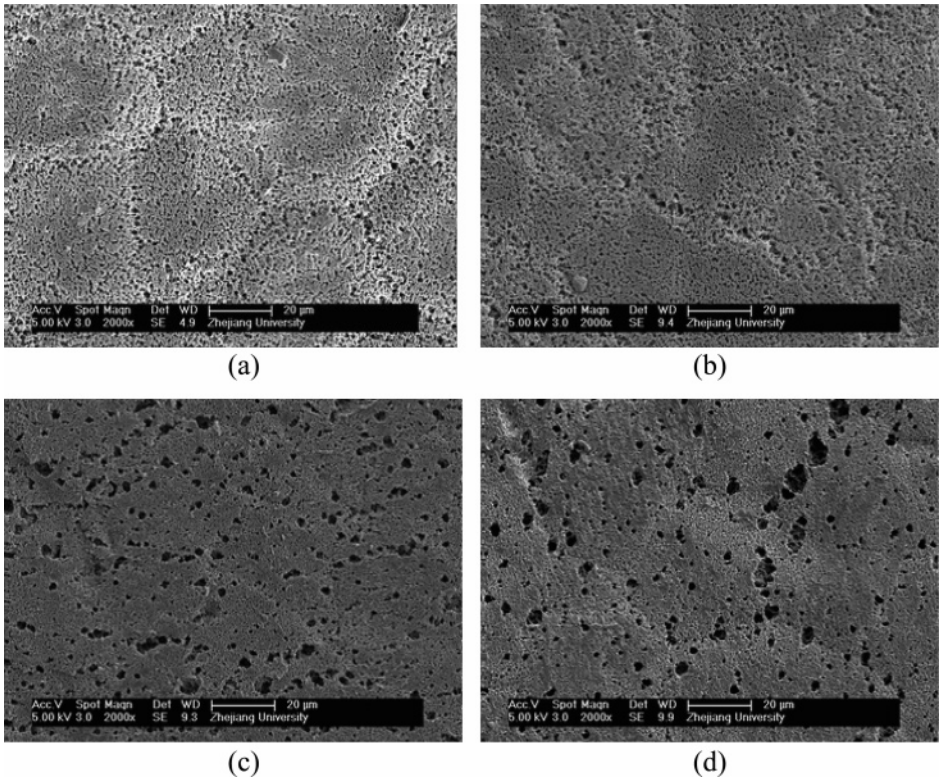


Figure 6. SEM images of the nascent and modified PPMMs: (a) 0, (b) 32.65, (c) 122.45, and (d) 146.94 μg/cm^2 AG-grafted PPMMs.

was found that the GD increased obviously with the UV irradiation time at first and then remained almost constant when the radiation time exceeded 25 min. This phenomenon was due to the fact that with the increase of the UV radiation time, more active sites were produced and more monomers were grafted onto the membrane surface, whereas all PPMMs were dipped into the photoinitiator solution for the same interval (60 min) and the adsorbed photoinitiator was approximately definite. Consequently, the active sites on the membrane surface could not increase infinitely; 20−25 min was enough and also ultimate for membrane surface activation. On the other hand, the monomers were consumed in the first 25 min, and increasing UV irradiation time did not result in additional grafting after 25 min.

The effect of BP concentration on the GD is shown in Figure 3. A series of initiator solutions (BP in *n*-heptane) with different concentrations was used, and the monomer concentration and UV irradiation time were fixed as 60 g/L and 25 min, respectively, for these experiments. As can be seen from Figure 4 , increasing the initiator concentration brought significant enhancement of GD because more active sites were generated. Subsequently, more glycopolymer chains were tethered on the membrane surface in the same reaction time (25 min) and higher GD was achieved. However, the increase in initiating sites on the membrane surface also increased the density of the propagating chain radicals, which led to intensive termination reactions and prevented further growth of the grafted chains. However, the adsorbed layer of photoinitiator at high-concentration cases also disturbed the contact of monomer and the substrate surface and accelerated the homopolymerization. Therefore, the GD decreased at high photoinitiator concentration.

Chemical and Morphological Changes of the Membrane Surface. Figure 4 is the FT-IR/ATR spectra of the nascent and AG-grafted PPMMs. The modified membranes showed a broad absorption band at 3290 cm^{-1}, and another peak at 1034 cm^{-1} (see the inset of Figure 4) was also found, which was attributed to the hydroxyl groups of the AG pendants.

To gain further information about the graft polymerization of AG on PPMMs, the chemical composition of the membrane surface was analyzed by XPS. Figure 5 shows the XPS spectra of unmodified and polyAG-tethered PPMMs. For the unmodified PPMM surface, a major emission peak at 284.7 eV ascribed to the binding energy of C_{1S} was found. However, an additional peak at 534.0 eV, which corresponded to the binding energy of O_{1S}, was detected from the spectrum of the polyAG-modified membrane surface. Moreover, the peak intensity of O_{1S} increased with the grafting density of AG.

In addition to chemical changes of the membrane surface, surface morphology of the modified PPMMs should also be considered. For this purpose, SEM pictures for the membrane surfaces with different grafting densities were taken. Figure 6 shows the typical images. As can be seen from Figure 6a, the nascent PPMM used in this study showed relatively high porosity and small pore size. With the increase of polyAG grafting density from 32.65 to 146.94 μg/cm^2, the membrane surface was covered by polyAG layer gradually and some larger pores can be observed in the images of higher grafting density cases which are not present in the lower grafting density ones (Figure 6b−d). With the coverage of the polyAG layer, the surface pores were blocked and the porosity decreased evidently. However, the grafted polyAG layer was asymmetric, and therefore, at areas without coverage of polyAG chains larger pores were found.

To study the hydrophilicity changes on the membrane surface, contact angle measurements were carried out. Commonly, contact angle measurements are the most convenient way to assess the changes in the wetting characteristics of the material surface. Figure 7 shows the static contact angle of PPMMs with different grafting densities. It was found that the static contact angle decreased greatly with the increase of AG grafting density. The static contact angle decreased evidently from about 149° to 80° with the increase of AG grafting density from 0 to 187.76 μg/cm^2. This decrease of the contact angle indicated that a relatively

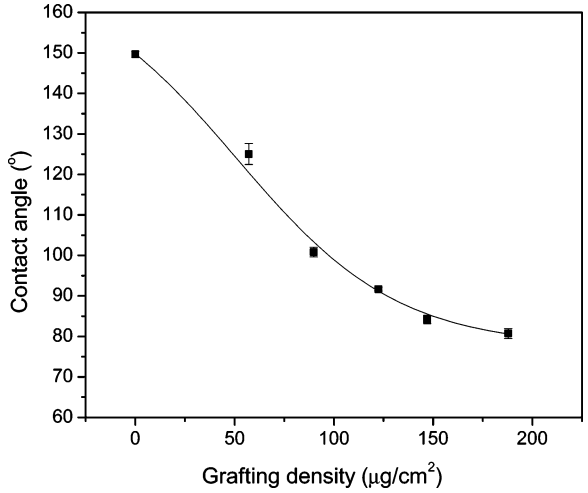


Figure 7. Effect of grafting density on the water contact angle.

hydrophilic surface was obtained by grafting AG onto the membrane surface. It was ascribed to the contribution of hydroxyl groups in AG moieties. The contact angle of the nascent PPMM used in this study was higher than general ones (about 100−120°) because of the relatively high surface porosity or, accordingly, roughness.[23] However, compared with our previous study,[24] a relatively high contact angle was obtained. This might be ascribed to the fact that sugar moieties with an open-chain structure were more efficient in improving the hydrophilicity of the polymeric material surface than those with cyclic ones.[18]

Lectin Recognition. Lectins are a large group of carbohydrate binding proteins and play key roles in various biological processes. The biological activities of lectins are greatly dependent on the carbohydrate binding properties, which are achieved by the specific recognition between lectins and carbohydrates. On the other hand, the mechanism of these interactions between carbohydrates and lectins is still not well known and attracts much interest. In consequence, synthetic carbohydrate-based polymers, "glycomimics", are emerging as important well-defined tools for investigating carbohydrate−protein interaction.[5,25] In this work, concanavalin A, the first found and most investigated lectin which specifically recognizes α-glucopyranoside and α-mannopyranoside residues with free 3-, 4-, and 6-hydroxyl groups, was used as the model protein to evaluate the recognition property of the tethered polyAG chains. Figure 8 shows the typical results. For the AG-grafted PPMMs with lower GD, weak interactions were observed, which was ascribed to the low affinity ($K_a = 10^3$−10^4 M^{-1}) between simple glucoside moieties and Con A. However, when the sugar density on the surface exceeded a critical value (about 90 μg/cm^2), the interactions increased obviously as a result of the "glycoside cluster effect"; therefore, Con A adsorbed to the membrane surface dramatically. On the other hand, almost no interactions were detected without $MnCl_2$, $CaCl_2$, and NaCl in PBS. Mn^{2+} and Ca^{2+} are two ions essential for the saccharide-binding activity of Con A,[26] while NaCl stabilizes Con A in solution.

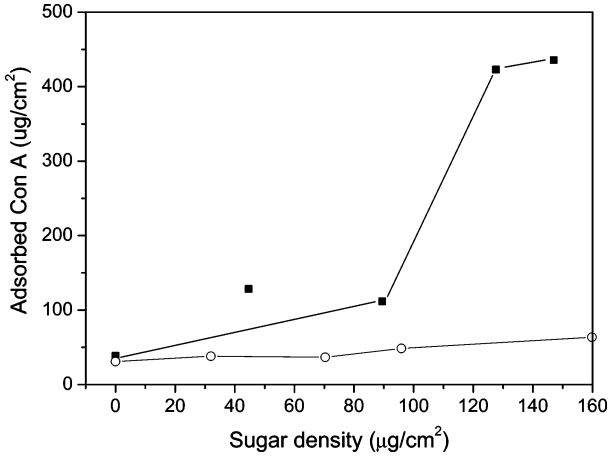


Figure 8. Amount of Con A adsorbed to the PPMM surface with different sugar densities: (○) in PBS and (■) in PBS containing 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$, and 0.1 M NaCl.

Conclusions

A glycopolymer-tethered porous surface was achieved by a UV-induced photografting process (BP as photoinitiator) of AG. The sugar density on the surface could be adjusted by adjusting monomer concentration, UV irradiation time, as well as photoinitiator concentration. Modification of the surface was confirmed by FT-IR/ATR, XPS, and SEM. Moreover, a decrease of the contact angle also demonstrated the grafting of this hydrophilic monomer. Con A was adsorbed to this surface specifically by formation of complexes between the glucose residues of tethered glycopolymer chains and Con A in the PBS (pH = 7.36). Membrane surfaces with lower sugar densities showed weak interactions with Con A. However, the critical effect of the sugar ligand density on the Con A adsorption amount was revealed when the sugar density was increased continuously. On the other hand, Mn^{2+} and Ca^{2+} were essential for the recognition event of glucose and Con A, and without these two ions almost no adsorption of Con A was detected. One can envisage that, combined with the separation ability, this surface-glycosylated membrane would be very promising in protein separation and drug delivery systems. Moreover, the described glycosylation strategy can also be extended to other polymer materials such as nanoparticles and nanofibers, which are prospects for obtaining very high-valency glycoligands.

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(23) Patankar, N. A. *Langmuir* **2003**, *19*, 1249−1253.
(24) (a) Yang, Q.; Xu, Z.-K.; Dai, Z.-W.; Wang, J.-L.; Ulbricht, M. *Chem. Mater.* **2005**, *17*, 3050−3058. (b) Yang, Q.; Xu, Z.-K.; Hu, M.-X.; Li, J.-J. Wu J. *Langmuir* **2005**, *21* (23), 10717−10723.
(25) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674.
(26) Nakamae, K.; Miyata, T.; Jikihara, A.; Hoffman, A. S. *J. Biomater. Sci. Polym. Ed.* **1994**, *6*, 79−90.