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Novel Photoinduced Grafting−Chemical Reaction Sequence for the Construction of a Glycosylation Surface

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Carbohydrates play a major role in many recognition events, such as blood coagulation, immune response, fertilization, cell growth, embryogenesis, and cellular signal transfer, which are essential for the survival of living entities. Synthetic carbohydrate-based polymers, so-called glycopolymers, are emerging as important well-defined tools for investigating carbohydrate-based biological processes and for simulating various functions of carbohydrates. In this work, we present a facile strategy for the formation of glycopolymer tethered on polypropylene microporous membrane surface. Acrylamide was grafted onto the polypropylene microporous membrane surface by photoinduced graft polymerization in the presence of benzophenone. The amide groups of grafted poly(acrylamide) were then transformed to primary amine groups by the Hofmann rearrangement reaction. Quantificational evaluation of the rearrangement reaction was carried out by ninhydrin method and mass weighting. Sugar moieties were coupled with the grafted functional layer to form glycopolymer by the reaction between primary amine groups and carbohydrate lactones. The grafting of acrylamide, the conversion of amide groups to amine groups, and the coupling of sugar moieties were confirmed by Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy combined with surface morphology observation by scanning electron microscopy.

Introduction

Carbohydrates are ubiquitous in living entities and have been found on the surface of nearly every cell in the form of polysaccharides, glycoproteins, glycolipids, or/and other glycoconjugates.[1,2] They serve as sites for docking other cells, molecules, and pathogens in a more or less specific recognition process, which is eventually triggered by carbohydrate−protein interactions.[3] Though the mechanisms and the structures of carbohydrates are still unknown, these carbohydrate−protein interactions are 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.[4]

Recently, there has been great interest in the carbohydrate−protein interactions, and many efforts have been made to reveal the underlying essence. However, the weak affinity of the interaction has hindered attempts to develop a comprehensive understanding of carbohydrate functions.[5,6] Indeed, the carbohydrate-binding proteins in nature typically aggregate into higher-order oligomeric structures, which suggest that the binding limitations can be circumvented through multivalency. The binding strength and also its specificity are remarkably improved by multivalent interactions, which are found quite regularly in biosystems.[7] The mechanism by which multivalent ligands act is still not very well-known, but it is increasingly accepted that the "cluster glycoside effect" relies on aggregation.[8] Consequently, a large number of different synthetic multivalent glycoligands (such as glycoclusters, glycodendrimers, and glycopolymers, etc.) have been designed to interfere effectively with the carbohydrate−protein interactions and to facilitate the investigation of the multiple interactions occurring during these molecular recognition events.[9] Among these man-made multivalent carbohydrate ligands, glycopolymers continue to be attractive because of very high valency, the ease in controlling the molecular structure, and the facile ability to vary the species of the sugar ligands. In the past decade, a great number of sugar-containing monomers and their polymers have been synthesized as reviewed by Wang et al.,[2] Ladmiral et al.,[7] and Okada et al.[10] On the other hand, these polymers are expected to display complex functionalities, similar to those of natural glycoconjugates, which might be able to mimic, or even exceed, their performance in specific applications.[11] However, bulk glycopolymers often take the disadvantage of great steric hindrance.[6,12] The intertwists of the polymer chains result in relatively low effective sugar residues for the 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.[13]

In our previous works, glycopolymers were tethered on the polypropylene microporous membrane (PPMM) surface by the

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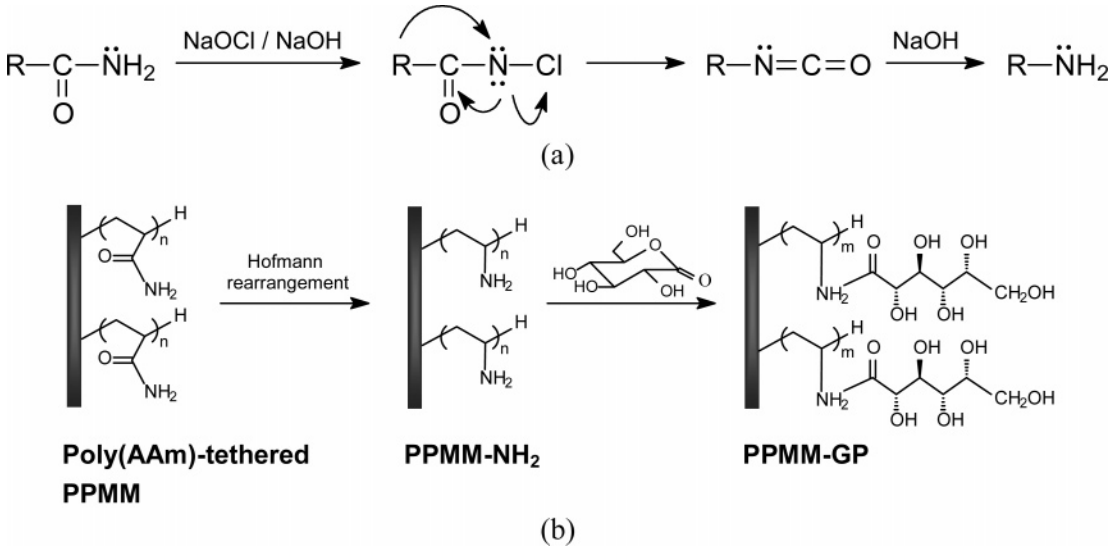
Scheme 1. Schematic Diagrams Illustrating the Hofmann Rearrangement Reaction (a) and the Whole Processes for the Formation of Glycosylated Surface (b)



graft polymerization of glycomonomer[14] and polymer analogous reaction.[15] Nevertheless, most glycomonomers have to be synthesized in a protected way to avoid unwanted side reactions in the hydroxyl groups and are comparatively difficult to separate and purify from other chemicals, especially when the sugar moiety is disaccharide or oligosaccharide. On the other hand, these monomers showed relatively low activity in the graft polymerization processes, and, subsequently, low surface sugar density was obtained.[14] For achieving clustered structure and for further applications in protein recognition and purification, high sugar moiety density is necessary. Acrylamide (AAm) is a commercially available monomer with high activity for graft polymerization. Several studies have reported the surface modifications by grafting AAm to improve the hydrophilicity and antifouling property of various substrates.[16−20] Although derivatization can be obtained by reactions with aliphatic diamines[21,22] and hydrazide[21] or coupling reactions with glutaraldehyde,[23,24] the amide groups of poly(AAm) are not very reactive. Hofmann rearrangement reaction gives a chance by transforming the amide groups to primary amine groups, which are more preferable and versatile for further reactions.[25−28] In this work, UV-induced graft polymerization was employed to tether poly(AAm) onto the PPMM surface in the presence of benzophenone and very high grafting density (GD, ~34 μmol/cm^2; see Supporting Information) was achieved compared with our previous results using other monomers.[14,15] Then, the amide groups were transformed into primary amine groups by Hofmann rearrangement reaction. Sugars in D-gluconolactone form were reacted with the amine groups and coupled to the polymer backbone to generate glycopolymer (see Scheme 1). With the sugar moieties (glycopolymer) on the surface, we conceive this porous support (membrane) can serve as lectin or antibody-binding assay,[29−31] chromatographic support for affinity chromatography and for the isolation of proteins with specificity via different sugar residues,[32,33] molecularly imprinted polymer,[34,35] and enzyme immobilization support.[36−38]

Experimental Section

Materials. The PPMMs were 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 a relatively high porosity about 75−80%. All the membranes used in this study were cut into rotundity with a diameter of 3.95 cm (area = 12.25 cm^2). Before the graft polymerization, the membranes were dipped in acetone for 0.5 h and then rinsed with acetone for several times to remove any impurity adsorbed on the surfaces. After being dried in a vacuum oven at 40 °C for 1 h, these membranes were stored in a desiccator. Acrylamide (AAm), benzophenone (BP), *n*-heptane, sodium hydroxide, and D-gluconolactone were analytical grade and were used without further purification. Sodium hypochlorite (5 wt % aqueous solution) was a commercial product and was used as received. All water used was deionized and ultrafiltrated to 18 MΩ.

Preparation of PPMM-NH$_2$. Grafted poly(AAm) layer was generated by UV-induced graft polymerization of AAm on the membrane surface in the presence of benzophenone (see Supporting Information). The amide group on poly(AAm)-tethered PPMM

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surface was converted to primary amine groups by Hofmann rearrangement reaction following the procedures illustrated in Scheme 1b. Detailed processes can be found elsewhere.[28] Briefly, sodium hydroxide solution was added to sodium hypochlorite solution dropwise at 0 °C with vigorous stirring to give a reaction reagent. PPMM-AAm was immersed in this mixture and stirred for 1 h at 0 °C. After stirring at 25 °C for another hour, the membrane was rinsed with water and dried under vacuum at 40 °C.

Quantificational Evaluation of the Rearrangement Reaction. The amount of the primary amine groups on the surface was evaluated by ninhydrin method[39] and mass weighting. Ninhydrin method was used as a contrast, and all calculations were based on data obtained by mass weighting method.

For the mass weighting method, the amount of grafted AAm (μmol) was calculated by following equation:

$$M_{\mathrm{AAm}} = \frac{W_1 - W_0}{71}$$

and the quantity of the primary amine groups was calculated from weight variation before and after Hofmann rearrangement reaction as

$$M_{\mathrm{NH_2}} = \frac{W_1 - W_2}{71 - 43}$$

Thus the density of NH_2 was defined as

$$D_{\mathrm{NH_2}} = \frac{M_{\mathrm{NH_2}}}{A_m}$$

where W_0, W_1, and W_2 are the masses of the nascent membrane, the membrane after graft polymerization of AAm, and the membrane after Hofmann rearrangement reaction, respectively. The molecular weight of the repeat unit of grafted poly(AAm) chains is 71, and 43 presents the molecular weight of the repeat units on the grafted chains after the rearrangement reaction. A_m is the surface area of the PPMM. Here, for the convenience of calculation, we ignored the weight loss caused by the chain cleavage from the surface as side reaction.

The efficiency of the Hofmann rearrangement reaction was denoted by the ratio of the resulted primary amine groups to the grafted AAm:

$$R_{\mathrm{Hofmann}} = \frac{M_{\mathrm{NH_2}}}{M_{\mathrm{AAm}}}$$

Glycosylation of the PPMM-NH_2. Sugar moieties were bound onto the membrane surface by the reaction between the primary amine groups of the PPMM-NH_2 and D-gluconolactone. PPMM-NH_2 was put in a D-gluconolactone solution (50 mL, 0.2 M), and the mixture was shaken in a flask for 24 h at 25 °C. Then the membrane was washed with water with drastic use of a vibrator and dried under vacuum at 40 °C. The amount of sugar binding to the surface was calculated as

$$M_{\mathrm{sugar}} = \frac{W_3 - W_2}{178}$$

and the binding density (BD, μmol/cm^2) was defined as

$$BD = \frac{M_{\mathrm{sugar}}}{A_m}$$

where W_3 is the mass of the glycosylated membrane and 178 is the molecular weight gained by the coupling of the sugar moiety.

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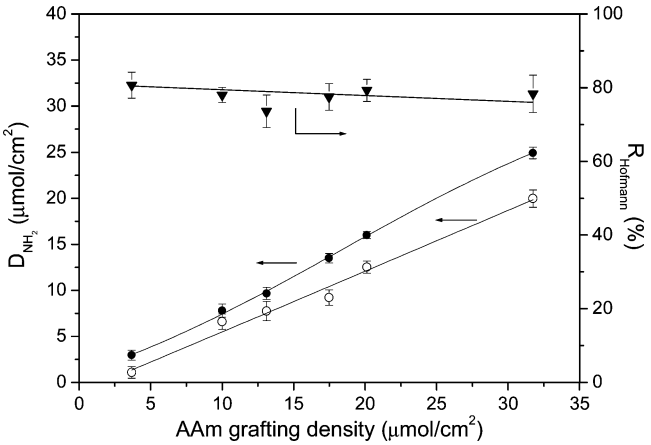

Figure 1. Effects of AAm grafting density on the density of primary amine group (data from (●) mass weighting and (○) ninhydrin method) and the efficiency of the Hofmann rearrangement reaction (▼).

Reaction Ratio of the Primary Amine Groups. The binding of sugar moieties and/or the formation of glycopolymer on the membrane surface was based on the reaction shown in Scheme 1. The amount of primary amine groups on the membrane surface used in this calculation was obtained by mass weighting method as mentioned above.

The amount of reacted primary amine groups which was also equal to the amount of sugar moieties in the glycopolymer was obtained. Thus, the reaction ratio of the primary amine group was

$$R_{\mathrm{NH_2}} = \frac{M_{\mathrm{sugar}}}{M_{\mathrm{NH_2}}}$$

Characterization. To investigate the varieties in surface chemical structure and morphology before and after the modification, the rearrangement reaction, and to confirm the glycopolymer formation, several 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°). For each spectrum 16 scans were taken 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, USA) with Al Kα radiation ($h\nu$ = 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 at 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 (from 0 to ~1200 eV) and the core-lever spectra with much high resolution were both recorded. Binding energies were calibrated using the containment carbon (C_{1s}, 284.7 eV). The data analysis was carried out on the PHI-MATLAB software provided by PHI Corp. No radiation damage was observed during the data collection time.

Scanning electron microscopy (SEM) images were taken on a field emission SEM (SIRION, FEI, USA). For this purpose, samples were washed with water and ethanol and dried at room temperature, and then coated with a 20 nm gold layer before SEM analysis.

Results and Discussion

Hofmann Rearrangement Reaction of Grafted Poly(AAm). Figure 1 shows the amounts of primary amine groups ($D_{\mathrm{NH_2}}$) and the ratio of Hofmann reaction (R_{Hofmann}) obtained with different AAm GDs. In the present study, no effort was made to study the detailed experimental factors. Generally, high temperature brings fast reaction but accelerates side reactions and chain cleavage for this reaction. With these taken into consideration, all

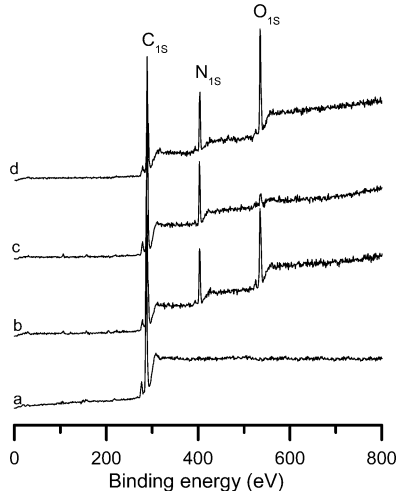

Figure 2. XPS spectra of the PPMM surfaces: (a) nascent; (b) poly(AAm)-tethered PPMM, GD = 25.87 μmol/cm^2; (c) PPMM-NH_2, D_{NH_2} = 16.47 μmol/cm^2; (d) PPMM-GP, BD = 5.23 μmol/cm^2.

experiments were carried out at 0 °C for 1 h and at 25 °C for another 1 h, which gave a fast reaction and relatively acceptable yield. At the same time, relatively low hypochlorite concentration (0.02 M) with excess of hydroxide ions (0.045 M) also restrained the chain scission.[25] As expected, D_{NH_2} increased almost linearly with the increase of AAm GD (Figure 1). On the other hand, the Hofmann rearrangement reaction of these AAm-grafted PPMMs with various GDs showed high efficiency between 75 and 82%. In our experiments, PPMMs with different AAm GDs were rearranged in the same reaction condition (time, temperature, and amount of sodium hypochlorite/sodium hydroxide). Thus, the reaction ratio of amide groups showed relative stability. However, besides primary amine groups, other functional groups were also encountered in the Hofmann rearrangement reaction of grafted AAm such as carboxyl, urea, and remaining amide groups.[40] Moreover, chain cleavage from the surface was inevitable in the reaction, especially at high temperature. The weight losses caused by chain cleavage were much lager than those caused by conversion to primary amine groups with equimolar amounts of amide groups. For these reasons, data from mass weighting (filled dots in Figure 1) were higher than those obtained by ninhydrin method[39] (open dots in Figure 1).

FT-IR/ATR and XPS were employed to detect the chemical changes of the modified PPMM. For the control PPMM surface (Figure 2a), a major emission peak at 284.7 eV ascribed to the binding energy of C_{1s} was found. However, additional peaks at 402.8 and 534.0 eV, corresponding to the binding energies of N_{1s} and O_{1s}, respectively, were detected from the spectrum of the poly(AAm)-tethered PPMM surface (Figure 2b). Its high-resolution spectrum corresponding to C_{1s} is shown in Figure 3a to distinguish the different types of functional groups on the surface. It was found that the C_{1s} spectrum could be resolved into two peaks. One peak at a binding energy of 284.7 eV was assigned to C−H and C−C. Another peak at a binding energy of 287.8 eV was ascribed to the C atoms in the amide groups of grafted AAm (O═*C*−NH_2). After the Hofmann rearrangement reaction, the intensities of the O_{1s} peak at 534.0 eV in the XPS spectrum (Figure 2c) as well as absorptions at 1654 and 1625 cm^{-1} in the FT-IR/ATR spectrum (Figure 4a) decreased obviously due to the conversion of amide groups to primary amine groups. Moreover, as can be seen from Figure 3b, the core lever C_{1s} spectrum of the PPMM-NH_2 surface showed a much more symmetrical peak than that of poly(AAm)-tethered PPMM. With the formation of primary amine groups on the PPMM surface, two fitted peaks at 284.7 and 286.5 eV, attributed to *C*−H and *C*−NH_2 respectively, were found. Remnant peaks in XPS (534.0 eV) and FT-IR/ATR (1654 and 1625 cm^{-1}) spectra were ascribed to the remaining amide groups and/or carboxyl and urea groups

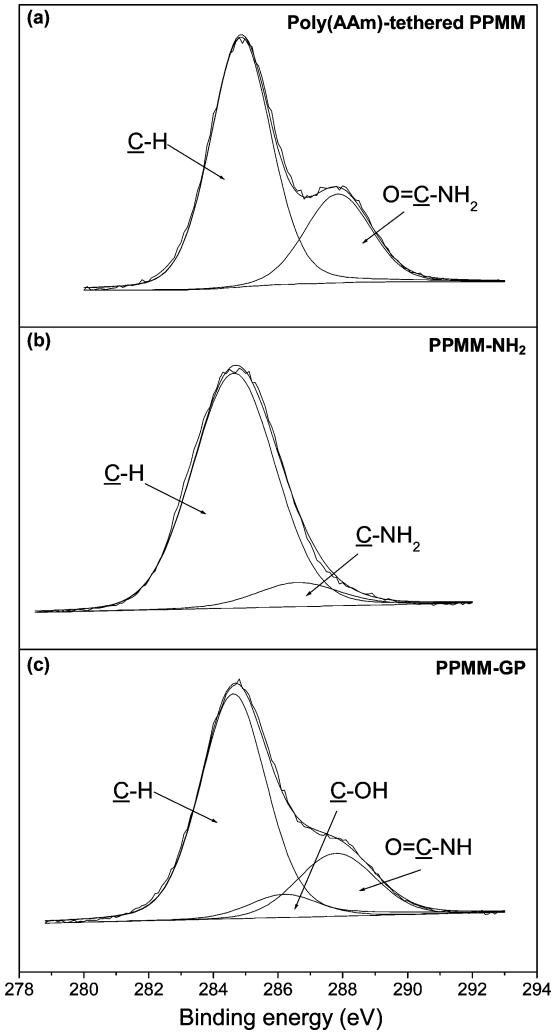

Figure 3. C_{1S} core-level spectra of poly(AAm)-tethered PPMM (a, GD = 25.87 μmol/cm^2), PPMM-NH_2 (b, D_{NH_2} = 16.47 μmol/cm^2), and PPMM-GP (c, BD = 5.23 μmol/cm^2) surfaces.

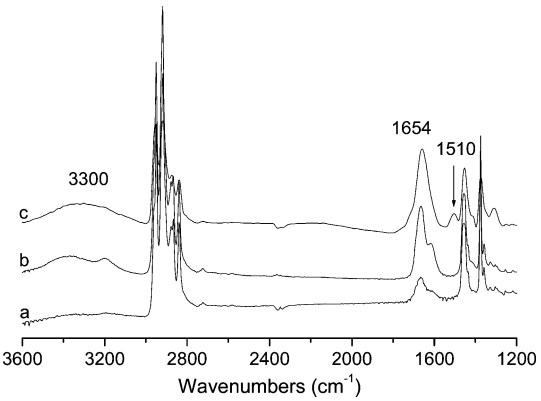

Figure 4. IR spectra of poly(AAm)-tethered PPMM (b, GD = 9.53 μmol/cm^2) suffered Hofmann rearrangement reaction (a, D_{NH_2} = 7.30 μmol/cm^2) and after sugar coupling (c, BD = 3.43 μmol/cm^2) surfaces.

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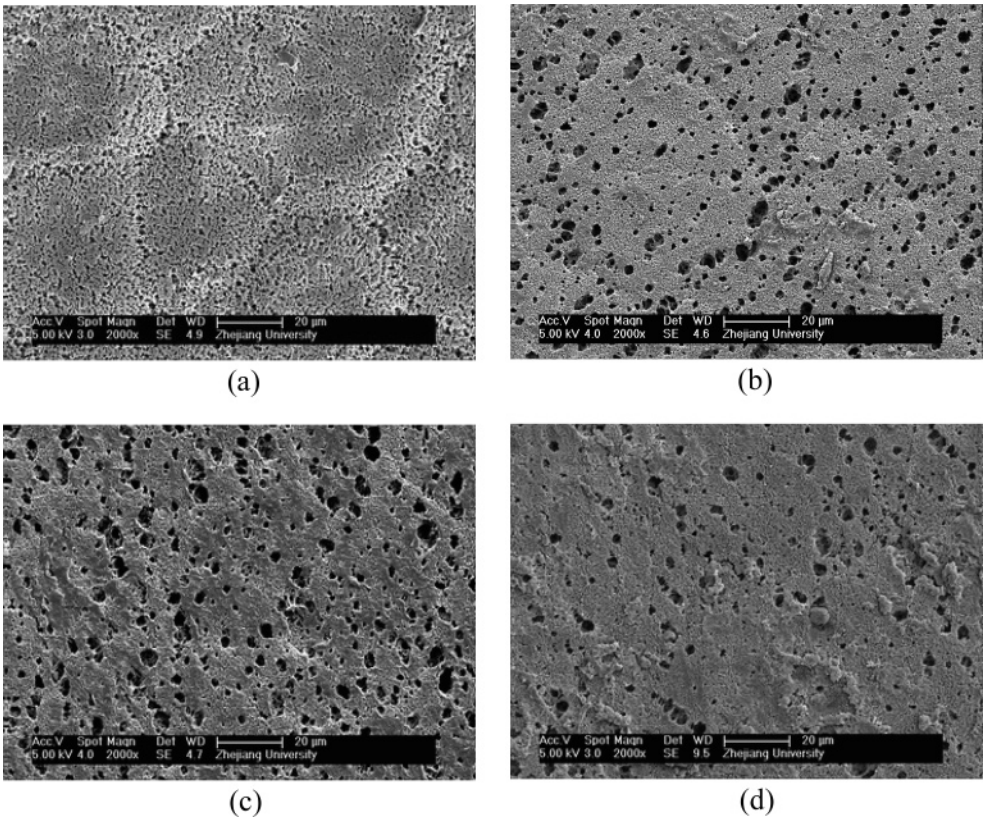

Figure 5. SEM images of the nascent PPMM (a), poly(AAm)-tethered PPMM (b, GD = 10.36 μmol/cm^2), PPMM-NH$_2$ (c, D_{NH_2} = 6.52 μmol/cm^2), and PPMM-GP (d, BD = 4.89 μmol/cm^2) surfaces.

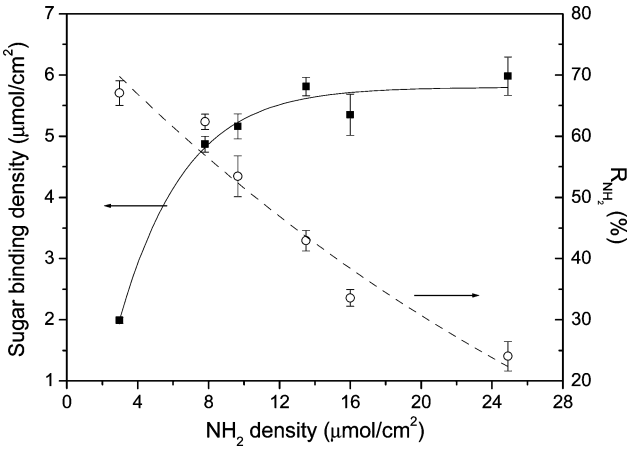

Figure 6. Influence of primary amine group density on the sugar binding density and the reaction ratio of the primary amine group.

generated by side reactions. After the graft polymerization of AAm, the membrane surface was covered by poly(AAm) chains and the porosity decreased evidently (Figure 5a,b). At the same time, some larger pores can be observed in the image of the poly(AAm) grafted membrane surface which are not present in that of the nascent PPMM. With the coverage of the poly(AAm) layer, the surface pores were blocked and the porosity decreased subsequently. However, the grafted poly(AAm) layer was asymmetric and, therefore, at the areas without coverage of poly(AAm) chains larger pores were found. However, from the SEM images in Figure 5b,c, no significant morphological change was observed before and after the Hofmann rearrangement reaction.

Glycosylation of the PPMM-NH$_2$. Figure 6 shows the influences of D_{NH_2} on the binding density (BD) of sugar moieties and the reaction ratio of primary amine groups (R_{NH_2}). The amount of attached sugar moieties increased with the D_{NH_2} because more binding sites were available on the surface and the reaction between the primary amine group and D-gluconolactone was promoted. However, as can be seen from Figure 6, the sugar BD turned to almost a constant when D_{NH_2} exceeded 10 μmol/cm^2. Furthermore, R_{NH_2} decreased obviously with the increase of D_{NH_2}. These results indicated that, in low-D_{NH_2} cases, the grafted chains showed relatively looser structure and higher surface vacancy. Reactant (D-gluconolactone in this work) could disperse facilely into the grafted layer for the coupling reaction. With the increase of GD, the grafted chains turned to a dense layer covered on the surface and the reactant could hardly diffuse in. Also, D-gluconolactone reacted with primary amine groups on the most outer surface of the grafted layer and formed large pendant groups on the tethered polymer chains. These pendant groups entwisted together and disturbed further reaction for D-gluconolactone with inner amine groups. As a result, only ~24% primary amine groups coupled with sugar moieties when D_{NH_2} rose to ~25 μmol/cm^2. Compared with our previous work,[15] much higher sugar density was obtained in this research. In the previous case, the amine surface was obtained by graft polymerization of 2-aminoethyl methacrylate (AEMA), and, however, the GD was low. AEMA showed much lower graft polymerization reactivity by contrast with acrylamide. On the other hand, lower GD brought looser structure of the grafted chains and, subsequently, relatively high reaction ratio was achieved.

With the coupling of the sugar moieties, the primary amine groups were converted into secondary amine groups, which brought the shift of the amide II peak from 1625 to 1510 cm^{-1} in the FT-IR/ATR spectrum (Figure 4c). In addition, a broad absorption band around 3300 cm^{-1}, assigned to −OH stretching vibration, was found for the PPMM-GP surface. Corresponding variation was also seen from XPS spectra. The most obvious result of the sugar coupling was the peak appearance of O_{1s} at 534.0 eV again in Figure 2d and, withal, the intensity was even

higher than that of poly(AAm)-tethered PPMM. This could be ascribed to the coupling of sugar moieties which contain many hydroxyl groups. The high-resolution C_{1s} peak of the PPMM-GP surface was fit with three unique carbon moieties (Figure 3c): *C*−H (284.7 eV), O═*C*−NH- (286.2 eV), and *C*−OH (287.7 eV). The additional peak at 286.2 eV was a signal from the C atom in the amide group (O═*C*−NH-). At the same time, from the SEM image of the PPMM-GP surface (Figure 5d), it was observed that a much denser glycopolymer layer was established on the membrane surface.

Conclusion

In summary, AAm was grafted onto the surface of a porous support, polypropylene microporous membrane, by UV-induced graft polymerization in the presence of benzophenone. The amide groups of tethered poly(AAm) could be converted into primary amine groups by Hofmann rearrangement reaction. Linear glucose was bound to these amine functionalized surfaces by the reaction between the primary amine groups and D-gluconolactone. FT-IR/ATR, XPS, and SEM analyses confirmed the establishing of the glycosylated surface and indicated that it was an effective way to tether glycopolymer chains to the support surface with high sugar density. In this study we focused on generation of the functional layer with the primary amine group and its reaction with a monosaccharide. Further work in coupling with disaccharide and their interaction against Con A has being carried out in our laboratory. Results will be reported in sequel paper. Also, one can extend this strategy to other kinds of monosaccharides, oligosaccharides, and even polysaccharides expediently.

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Supporting Information Available: Detailed AAm graft polymerization process and the influence of AAm GD on the static contact angle. This material is available free of charge via the Internet at http://pubs.acs.org.

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