Extension and Shrinkage of Polymer Brush Grafted onto Porous Membrane Induced by Protein Binding

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ABSTRACT: Two kinds of ionizable groups, namely, diethylamino and sulfonic acid groups, were introduced to a polymer brush by reaction of the epoxy groups of poly(glycidyl methacrylate) grafted onto a porous hollow-fiber membrane with diethylamine and sodium sulfite, respectively. Aminoacylase solution was permeated through the pores under a constant permeation pressure, and the changes in both the protein concentration of the effluent and the permeation pressure were continuously monitored. Aminoacylase was bound in multilayers to the polymer brush based on an ion-exchange interaction. The diethylamino-group-containing polymer brush extended with the capture of the protein, whereas the sulfonic acid-group-containing polymer brush shrank with the progression of protein binding.

Introduction

Polymer chains grafted onto a pore surface form a kind of polymer brush. Radiation-induced graft polymerization is a powerful method in that the polymer chains can be attached to various shapes of trunk polymers such as film,[1] hollow fiber,[2] and nonwoven fabric.[3] Ionizable polymer brushes are reported to bind proteins in multilayers.[4,5] This multilayer binding of proteins has practical advantages over the protein binding structure of conventional polymeric materials, e.g., agarose gels, used for protein purification in the pharmaceutical and food industries.

At present, direct characterization of the polymer brush is difficult because the grafted polymer chains cannot be easily isolated from the trunk polymers. Alternative methods are required to evaluate the static and dynamic properties of polymer brushes. The permeability of porous membranes with immobilized ionizable polymer brushes has been analyzed.[6−8] The fluorescence probe technique was used to investigate the conformational change of an amino-group-containing polymer brush grafted onto a porous membrane.[9] The quantitative analysis of the flux change with the progression of protein binding under a constant permeation pressure across the porous membrane is effective in elucidating the behavior of the ionizable polymer brush grafted onto the pore surface. Tsuneda et al.[10] demonstrated the multilayer binding of bovine serum albumin (BSA) to a polymer brush containing a diethylamino (DEA) group as an ionizable group by hydrodynamic analysis.

In this study, we prepared two types of ionizable polymer brushes: diethylamino-group ($-N(C_2H_5)_2$)- and sulfonic acid-group ($-SO_3H$)-containing polymer brushes grafted onto the pore surface of a porous hollow-fiber membrane. The amount of protein bound and the flux during the permeation of a protein solution through pores edged by the polymer brush were determined to enable discussion of the extension and shrinkage of the polymer brush. Here, aminoacylase was used as a model protein.

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Experimental Section

Materials. A commercially available hollow-fiber membrane, supplied by Asahi Chemical Industry Co., was used as the trunk polymer for grafting. This membrane has been used for microfiltration. The hollow fiber had inner and outer diameters of 1.8 and 3.1 mm, respectively, with an average pore diameter of 0.4 μm and a porosity of 70%.

Technical-grade glycidyl methacrylate (GMA) was purchased from Tokyo Kasei Co. and used without further purification. Aminoacylase (M_r 86 000) was purchased from Sigma Co. Other chemicals used were of analytical grade or higher.

Preparation of Ionizable Polymer Brush Grafted onto Pore Surface. The preparation scheme for two kinds of ionizable polymer brushes is illustrated in Figure 1: the polymer brush containing diethylamino or sulfonic acid groups as the ionizable groups was appended onto a porous hollow-fiber membrane via four steps.[11] First, the 6-cm-long hollow fiber was irradiated in a nitrogen atmosphere at ambient temperature with an electron beam using a cascade-type accelerator (Dynamitron model IEA 3000-25-2, Radiation Dynamics Inc., New York). The dose was set at 200 kGy. Second, the irradiated hollow fiber was immersed in a 10% (v/v) GMA/methanol solution that had previously been deaerated. After 10 min, the hollow fiber was removed and washed thoroughly with dimethylformamide to remove residual GMA and poly-GMA homopolymer. Third, some of the produced epoxy groups were converted into diethylamino groups ($-N(C_2H_5)_2$) or sulfonic acid groups ($-SO_3H$) by immersing the

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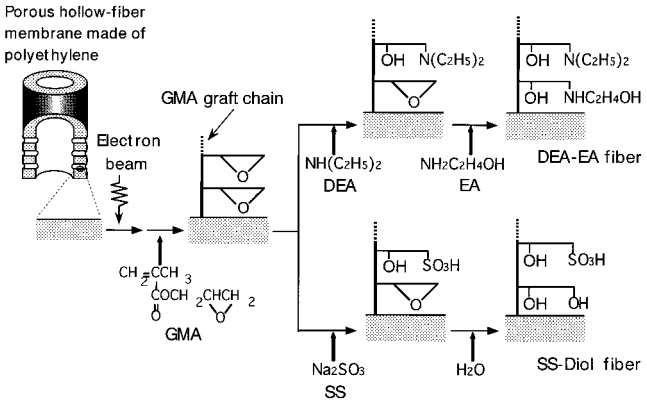

Figure 1. Preparation scheme for diethylamino- and sulfonic acid-group-containing porous membranes.

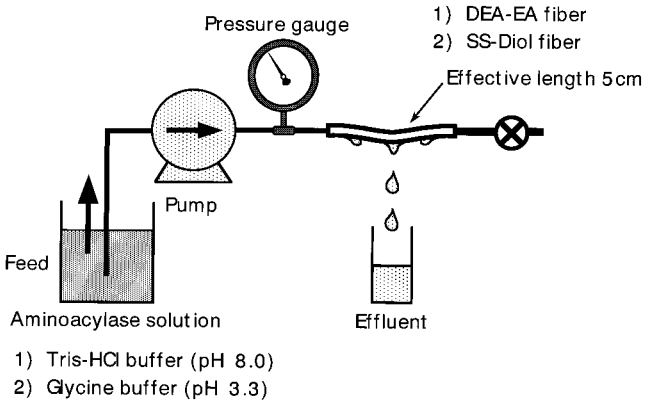

Figure 2. Experimental apparatus for protein binding during permeation of protein solution.

GMA-grafted hollow fiber in a mixture of diethylamine/water = 50/50 (volume ratio) or sodium sulfite/isopropyl alcohol/water = 10/15/75 (weight ratio), respectively. The conversion of the epoxy group to the ionizable groups, i.e., diethylamino (DEA) and sulfonic acid (SS) groups, was calculated from the weight gain of the hollow fiber due to the modification as follows:

$$\text{conversion} = 100\ (\text{moles of the ionizable group introduced})/(\text{moles of the epoxy group before modification}) = (W_2 - W_1)/(73 \text{ or } 82)/W_2 \quad (1)$$

where W_1 and W_2 are the weights of the GMA-grafted and ionizable-group-introduced hollow fibers, respectively. The figures 73 and 82 are the molecular weights of $NH(C_2H_5)_2$ and H_2SO_3, respectively. Finally, the remaining epoxy groups were reacted with ethanolamine at 313 K for 6 h and sulfuric acid at 353 K for 1 h to reduce the nonselective adsorption of proteins onto the hollow-fiber surface.[12] The resultant two kinds of hollow-fiber membranes with the immobilized polymer brushes containing diethylamino and sulfonic acid groups are referred to as DEA-EA and SS-Diol fibers, respectively.

Protein Binding during Permeation through Pores. The hollow fiber (effective length 5 cm) was positioned in a configuration, as shown in Figure 2. A 1.0 g/L aminoacylase solution, the pH of which was adjusted to 8.0 with 14 mM Tris−HCl buffer and 3.3 with 50 mM glycine buffer for DEA-EA and SS-Diol fibers, respectively, was forced to permeate radially outward through the pores of the hollow fiber. For the SS-Diol fiber only, 5.0 mM aqueous $MgCl_2$ solution was permeated prior to the permeation of the aminoacylase solution to improve the permeability via ionic cross-linking of the polymer brush.[13] Transmembrane pressure was measured under a constant permeation rate of 1.0 mL/min. The effluent

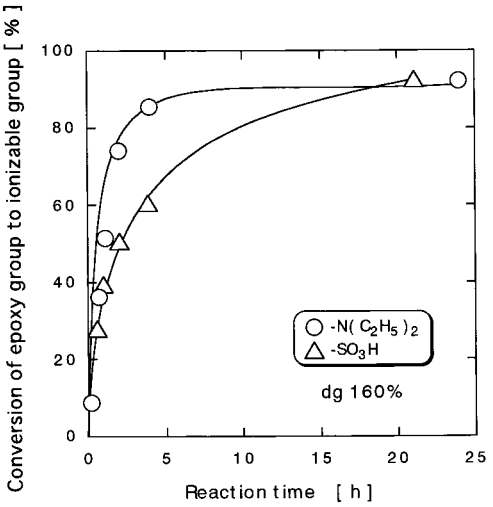

Figure 3. Time courses of conversion of epoxy groups to ionizable groups.

penetrating the outside surface of the hollow fiber was continuously collected in the fraction vials, and aminoacylase of each fraction was determined by measuring the UV absorbance at 280 nm. For comparison, a protein-free buffer solution was permeated across the membrane. The experiment was performed at ambient temperature.

The amount of protein adsorbed, q, was calculated as follows:

$$q = \int_0^{V_e} (C_0 - C)\, dV/W_3 \quad (2)$$

where C_0 and C are the protein concentrations of feed and effluent, respectively, and W_3 is the weight of the DEA-EA or SS-Diol fiber. V is the effluent volume, and V_e is the effluent volume where C reaches C_0.

The relative pore radius, r/r_0, was evaluated from the following equation based on Hagen-Poiseuille analysis:[8]

$$r/r_0 = (\Delta P_0/\Delta P)^{1/4} \quad (3)$$

Here ΔP is the permeation pressure, and the subscript 0 denotes the pressure for the protein-free buffer.

Results and Discussion

Properties of Porous Membrane Immobilizing Ionizable Polymer Brush. Glycidyl methacrylate was grafted onto the porous hollow-fiber membrane made of polyethylene, previously irradiated with an electron beam, at a degree of grafting of 160%: the weight of poly-GMA was 1.6-fold that of polyethylene. Time courses of the conversion of the produced epoxy groups to diethylamino or sulfonic acid groups are shown in Figure 3. The resultant hollow fibers with a conversion of 60% were used for protein binding, and the properties of the DEA-EA and SS-Diol fibers are summarized in Table 1.

Uniform distribution of chlorine and sulfur across the HCl-adsorbed DEA-EA and SS-Diol fibers, respectively, was determined by an X-ray microanalyzer, indicating that the ionizable polymer brush was appended uniformly throughout the membrane.

Multilayer Binding of Protein to Polymer Brush. Aminoacylase can bind to either diethylamino-group- or sulfonic acid-group-containing polymer brushes depending on the pH of the buffer solution. Breakthrough curves of aminoacylase dissolved in Tris-HCl buffer (pH 8.0) and glycine buffer (pH 3.3) for DEA-EA and SS-

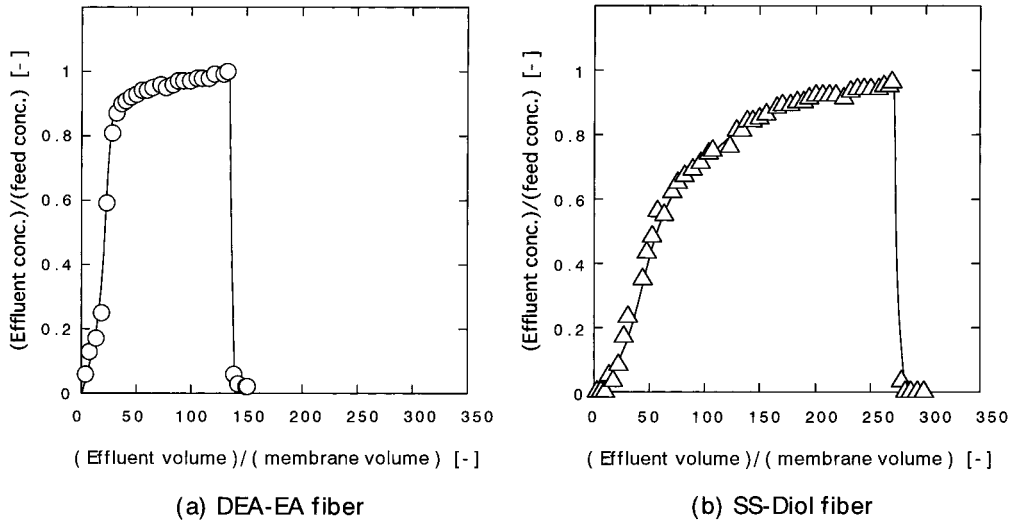

Figure 4. Breakthrough curves of aminoacylase: (a) DEA-EA fiber; (b) SS-Diol fiber.

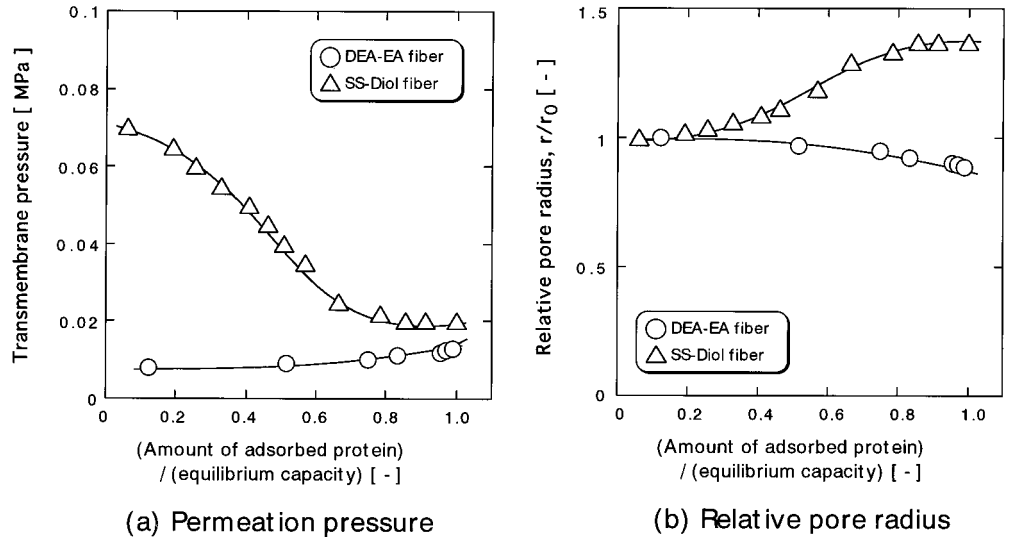

Figure 5. Changes in permeation pressure and relative pore radius: (a) permeation pressure; (b) relative pore radius.

Table 1. Properties of Porous Membranes with Immobilized Ionizable Polymer Brushes

	DEA-EA fiber	SS-Diol fiber
dg [%]	160	160
conversion [%]	61	60
ID [mm]	2.4	2.2
OD [mm]	4.4	4.2
density of ionizable group [mmol/g]	2.0	2.1
flux[a] [m/h]	1.8[b]	0.24[c]
specific surface area [m^2/g]	6.9	7.2

[a] Permeation pressure = 0.1 MPa; temperature = 298 K. [b] 14 mM Tris−HCl buffer (pH 8.0). [c] 50 mM Glycine buffer (pH 3.3).

Diol fibers, respectively, are shown in Figure 4. The amount of protein adsorbed, calculated using eq 2, was 92 and 430 mg per g of DEA-EA and SS-Diol fibers, respectively. This amount was converted to the degree of multilayer binding of the protein defined as

$$\text{degree of multilayer binding of protein} = \text{(amount of protein adsorbed)/(monolayer adsorption capacity)} \quad (4)$$

The monolayer adsorption capacity was theoretically calculated as

$$\text{monolayer binding capacity} = a_V M_r/(aN_A) \quad (5)$$

where a_v, a, M_r, and N_A are the specific surface area of the hollow fiber (6.9 and 7.2 m^2/g for DEA-EA and SS-Diol fibers, respectively), the area occupied by a protein molecule (7.4×10^{-17} m^2 for aminoacylase), the molecular mass of the protein (86 000), and Avogadro's number, respectively.

The permeation pressure change reflects the pore diameter change induced by protein binding to the polymer brush. The permeation pressure change with the progression of aminoacylase binding to DEA-EA and SS-Diol fibers is shown in Figure 5a. In this figure, the abscissa is the ratio of the amount of aminoacylase adsorbed to the amount of aminoacylase adsorbed at equilibrium; the ordinate is the ratio of the transmembrane pressure. The ordinate can be converted to relative pore radius using eq 3, as shown in Figure 5b. The pressure was clearly different between the two kinds of polymer brushes: the polymer brush containing weakly basic anion-exchange groups (diethylamino groups) captures protein while extending itself, whereas the polymer brush containing strongly acidic cation-exchange groups (sulfonic acid groups) entangles the protein forming a shrunken structure. The extension

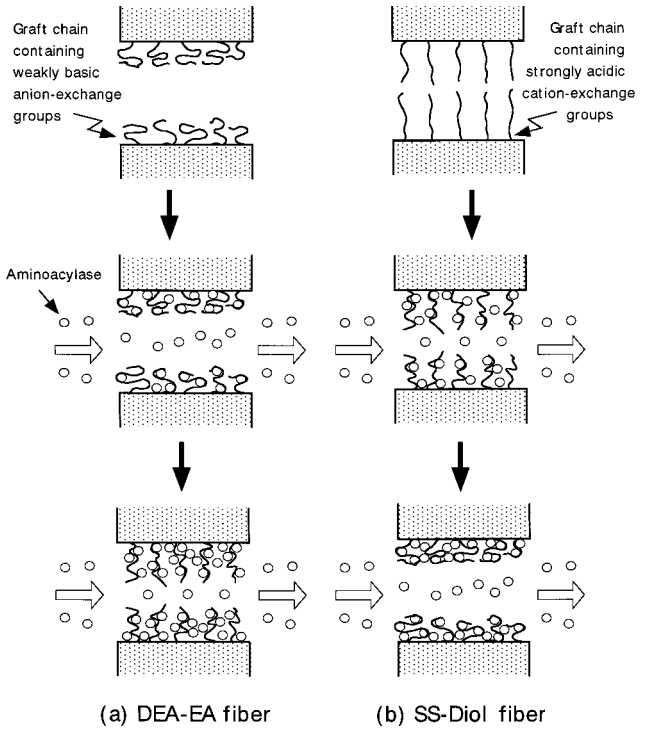

Figure 6. Extension and shrinkage of the ionizable polymer brush induced by protein binding.

and shrinkage of the polymer brush on the pore surface are schematically illustrated in Figure 6. The simultaneous determination of the amount of the adsorbed protein and the permeation pressure helps in understanding the dynamic behavior of the polymer brush.

Conclusion

Ionizable polymer brushes can form distinct conformational structures depending on the density of ionizable groups, and trap proteins in multilayers. Polymer brushes were immobilized onto the pore surface of porous hollow-fiber membranes by radiation-induced graft polymerization of epoxy group-containing monomers. Subsequently, epoxy rings were opened by diethylamine or sodium sulfite to introduce diethylamino (DEA) groups as weakly basic anion-exchange groups or sulfonic acid (SS) groups as strongly acidic cation-exchange groups, respectively. Aminoacylase solution was permeated through the pores edged by the ionizable polymer brushes. Changes in the transmembrane pressure measured at a constant permeation rate reflect the pore size change induced by protein binding to the polymer brush. The extension or shrinkage of DEA-group- or SS-group-containing polymer brushes, respectively, was demonstrated during binding of the protein to the polymer brush in multilayers. The analysis made in this study provides information regarding the conformation of the polymer brush.

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