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Solvent effect on protein binding by polymer brush grafted onto porous membranes

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Abstract

An epoxy-group-containing polymer chain was grafted onto the hollow-fiber form of a porous polyethylene membrane by the immersion of the electron beam-irradiated trunk polymer in glycidyl methacrylate diluted with methanol and 1-butanol. The epoxy group density ranged from 8.5 to 13.4 mol per kg of the trunk polymer. Subsequently, the epoxy groups produced were converted into sulfonic acid and diethylamino groups. The density of $-SO_3H$ and $-N(C_2H_5)_2$ groups was 0.40 and 2.2 mol per kg of the product, respectively. The polymer brush, defined as a polymer chain extending from the surface of a pore toward the interior of the pore, was evaluated from the determination of an equilibrium binding capacity of hen egg lysozyme (HEL) and bovine serum albumin (BSA). The polymer brush prepared in 1-butanol was found to be longer than that prepared in methanol from the determinations of liquid permeability and protein adsorptivity. The proteins were bound to the polymer brush prepared in 1-butanol, followed by the functionalization, at higher degrees of multilayer binding: about 30 for HEL and 6 for BSA.

Keywords: Polymer brush; Protein binding; Graft polymerization; Proteins; Lysozyme; Albumin

1. Introduction

Graft polymerization is a powerful technique to modify various forms of trunk polymers [1–3]. Polymer chains grafted onto a polymeric surface can exhibit excellent performance, for example, high capacity for capturing protein [4,5] and high activity for hydrolysis [6]. Irradiation by an electron beam or gamma ray, plasma, or photo will produce radicals as a starting site for grafting vinyl monomers.

Radiation forms the radicals throughout the trunk polymer because of its high energy, therefore, when a porous polyethylene is used as a trunk polymer, the formation site of the graft chains is classified into two, as illustrated in Fig. 1: graft chains embedded in the matrix, and graft chains extending from the pore surface. The former chains allow the matrix to swell, and the latter chains, referred to as a polymer brush, acquire the mobility of expansion and shrinkage in

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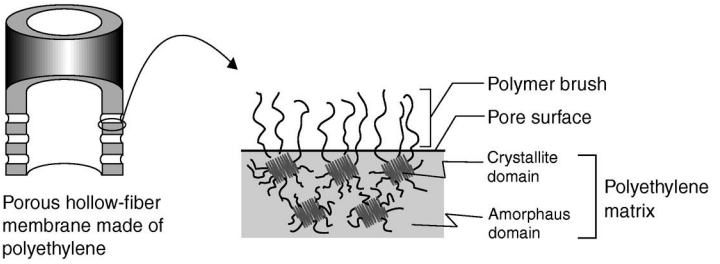


Fig. 1. Definitions for polymer brushes grafted onto the pore surface of a porous hollow-fiber membrane.

response to the environmental variations such as pH, ionic strength and temperature.

The control of the formation site of graft chains is essential in designing functional materials precisely. For example, metal ions diffuse easily into the matrix embedding the graft chains [7], whereas proteins interact only with the polymer brush because of their large sizes. Therefore, controlled formation of the polymer brush contributes to the realization of the high-performance of adsorbents capable of capturing proteins while maintaining feasible permeability of the protein solution. No attempt has been reported to control the length of the polymer brush grafted onto the porous polymeric membrane.

We have reported that the charged polymer brush, i.e. polymer brush containing the ion-exchange groups, held proteins in multilayers. For example, the polymer brush containing a diethylamino group as the positively charged group captured bovine serum albumin in eleven layers [5]. This can be explained by that the polymer brush extends from the pore surface toward the pore interior due to mutual electrostatic repulsion and provides a three-dimensional binding space for proteins. Protein multilayering leads to protein capturing of the adsorbents at high capacity.

The objective of this study was twofold: (1) to append the polymer brush onto the irradiated porous hollow-fiber membrane made of polyethylene by varying the solvent of the monomer, and (2) to experimentally verify the controlled formation of the polymer brush in terms of membrane swelling, degree of multilayer binding of proteins, and liquid permeability. Here, an epoxy-group-containing graft chain was selected as the graft chain, and subsequently the epoxy group was converted into sulfonic acid and diethylamino groups for capturing hen egg lysozyme and bovine serum albumin, respectively.

2. Experimental

2.1. Materials

A porous hollow-fiber membrane made of polyethylene was used as a trunk polymer for grafting. This hollow fiber had inner and outer diameters of 1.8 and 3.1 mm, respectively, with an average pore diameter and porosity of 0.4 μm and 70%. Glycidyl methacrylate (GMA, CH_2=$CCH_3COOCH_2CHOCH_2$) was purchased from Tokyo Kasei and used without further purification. Hen egg lysozyme (HEL, M_r 14 700, p*I* 10.7) and bovine serum albumin (BSA, M_r 67 500, p*I* 4.9) were purchased from Sigma. Other chemicals were of analytical grade or higher.

2.2. Grafting of epoxy-group-containing polymer chains

An epoxy-group-containing monomer was grafted onto a porous hollow-fiber membrane by a pre-irradiation technique: first, hollow-fiber membranes 10 cm long made of polyethylene were irradiated with an electron beam in a nitrogen atmosphere at ambient temperature. The dose was 200 kGy. Subsequently, the irradiated hollow fiber was immersed in a GMA–solvent (10:90, v/v) solution in a reaction ampoule. Here, methanol and 1-butanol were selected as a solvent. After a prescribed reaction time ranging up to 25 min at 313 K, the hollow-fiber membrane was taken out of the reaction ampoule and

washed with N,N-dimethylformamide (DMF). The GMA-grafted hollow-fiber membrane was dried under reduced pressure and weighed. The degree of GMA grafting was defined as follows:

$$dg = 100\,(W_1 - W_0)/W_0 \quad (1)$$

where W_0 and W_1 are the masses of the trunk and GMA-grafted hollow-fiber membranes, respectively. The resultant hollow-fiber membrane was referred to as a GMA-grafted fiber.

2.3. Introduction of charged groups to polymer brush

The epoxy groups produced were converted into two kinds of charged groups, i.e. sulfonic acid and diethylamino groups, to capture proteins based on an electrostatic interaction. A scheme for introducing two charged groups into the polymer brush is shown in Fig. 2. Reaction conditions were according to Kawai et al. [8]. The resultant porous hollow-fiber membranes containing the $-SO_3H$ and $-N(C_2H_5)_2$ groups were referred to as an SS fiber and a DEA fiber, respectively. Here, the conversion, x, was defined by the following:

$$x = 100\,[(W_2 - W_1)/(82 \text{ or } 73)]/[(W_1 - W_0)/142] \quad (2)$$

where W_2 is the mass of the dried SS or DEA fiber. The numbers 82 and 73 are the molecular masses of H_2SO_3 and $NH(C_2H_5)_2$, respectively. The remaining epoxy groups on the SS and DEA fibers were reacted with water and ethanolamine, respectively, to minimize nonselective adsorption of proteins on the polymer brush [9]. The resultant hollow-fiber membranes were referred to as SS-Diol (M or B, dg, x) and DEA-EA (M or B, dg, x) fibers, respectively. The letters M and B designate methanol and 1-butanol used as the solvent for GMA, respectively.

2.4. Determination of membrane properties

The swelling ratio of the length of the GMA-grafted, SS-Diol and DEA-EA fibers to the trunk fiber was measured in a wet state. The permeability was defined by dividing the permeation rate of a liquid by the inside surface area of the hollow-fiber membrane. For the SS-Diol fiber, prior to the measurement of the permeation rate, ionic cross-linking of the graft chains with magnesium ion (Mg^{2+}) was achieved by permeation of a 0.005 M aqueous solution of $MgCl_2$ to improve the permeability [10]. The permeation experiments were performed at a permeation pressure of 0.1 MPa and ambient temperature.

To determine the distribution of the charged

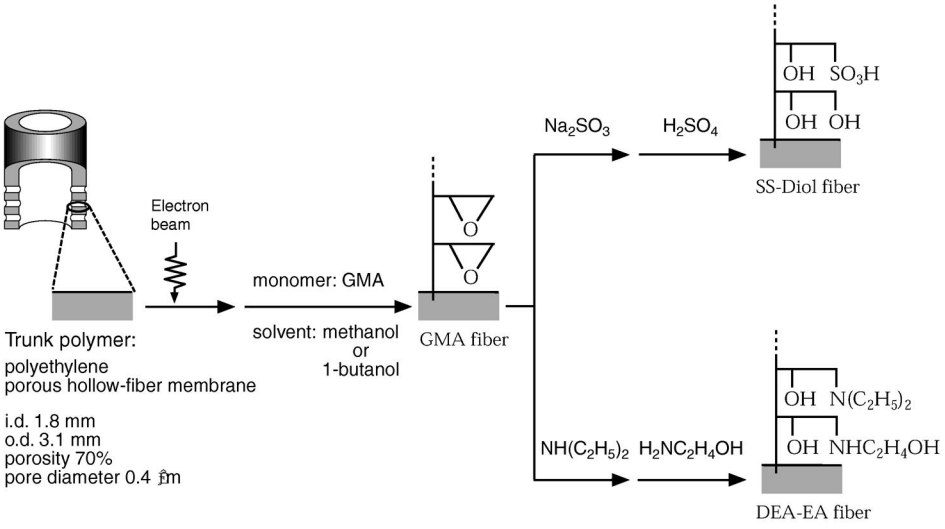


Fig. 2. Introduction of two charged groups into the polymer brush.

groups across the hollow-fiber membrane, the intensities of peaks in the micro-FT-IR of the SS-Diol and DEA-EA fibers were determined throughout their membrane thickness using a micro-FT-IR spectrophotometer (Perkin-Elmer, spectrum one autoimage system) in the attenuated total reflectance mode. The aperture size was 100×100 μm^2, and the measurement region was scanned in a 100-μm step.

2.5. Protein binding in the permeation mode

An SS-Diol or a DEA-EA fiber about 5 cm long was positioned as shown in Fig. 3. A 0.50 g/l HEL solution buffered with 0.0125 *M* carbonate buffer (pH 9.0) and a 5.0 g/l BSA solution buffered with 0.02 *M* Tris–HCl buffer (pH 8.0) were allowed to permeate radially outward from the inside surface through the SS-Diol and DEA-EA fibers, respectively, at a constant permeation pressure of 0.1 MPa and ambient temperature. The effluent penetrating the outside surface of the fiber was continuously collected in fraction vials and its protein concentration was determined by measuring the UV absorbance at 280 nm. The equilibrium binding capacity (EBC) was calculated by integrating the concentration difference between the feed and effluent as follows:

$$\mathrm{EBC} = \int_0^{V_e} (C_0 - C)\, \mathrm{d}V/W \tag{3}$$

where V, V_e, and W are the effluent volume, the effluent volume where C reached C_0, and the mass of the membrane, respectively. The degree of protein multilayer binding was defined as follows:

$$\text{Degree of protein multilayer binding} = \frac{\text{EBC}}{\text{Theoretical monolayer binding capacity}} \tag{4}$$

Theoretical monolayer binding capacity,

$$q_t = (a_v M_r)/(a N_{av}) \tag{5}$$

where a_v and a are the specific surface area of the fiber and the occupied area of a protein molecule, respectively. The value of a_v was determined using Quantasorb (Yuasa Ionics) according to the BET method. The values of q_t of HEL and BSA are 12 [11] and 58 mg/g [12], respectively. The terms M_r and N_{av} are molecular mass of the protein and Avogadro's number, respectively.

After the equilibration, the feed was switched from the protein solution to a protein-free buffer to wash the pores, and subsequently 0.50 *M* NaCl was allowed to permeate to elute the adsorbed protein. The elution percentage was evaluated as follows:

$$\text{Elution percentage} = \frac{100\ (\text{amount eluted})}{(\text{amount adsorbed}) - (\text{amount washed})} \tag{6}$$

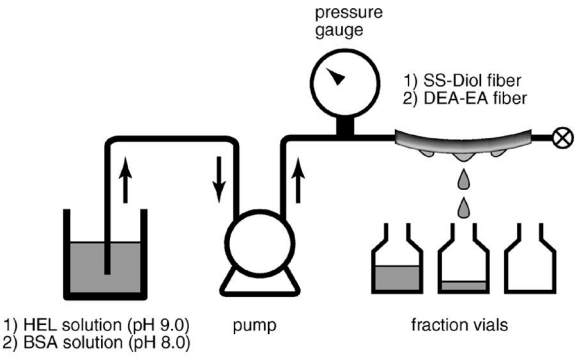


Fig. 3. Experimental apparatus for protein binding during permeation of protein solution.

3. Results

3.1. Preparation of charged polymer brushes

A time course of the degree of glycidyl methacrylate (GMA) grafting onto the hollow-fiber form of a porous polyethylene membrane is shown in Fig. 4. For example, the mass of 0.072 g of a 5-cm long original hollow fiber increased to 0.209 g after GMA graft polymerization in methanol; the degree of GMA grafting was calculated to be 190% using Eq. (1). The grafting rate of GMA in methanol as a solvent was higher than that in 1-butanol. Subsequently, charged groups were introduced to the poly-GMA graft chain via the addition of Na_2SO_3 and $NH(C_2H_5)_2$ to the epoxy groups. Conversions of the epoxy group into the sulfonic acid and diethyl-

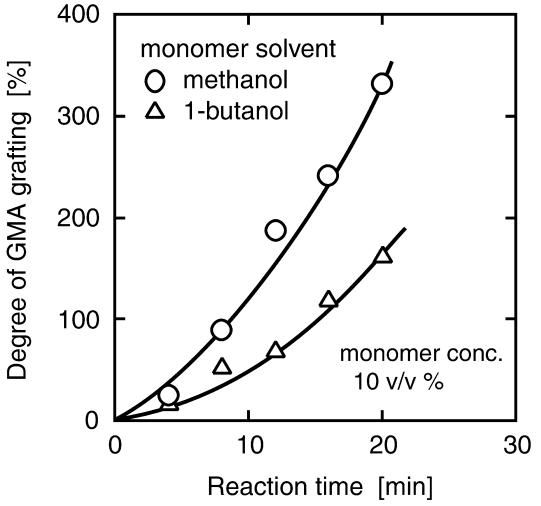


Fig. 4. Grafting rate of glycidyl methacrylate.

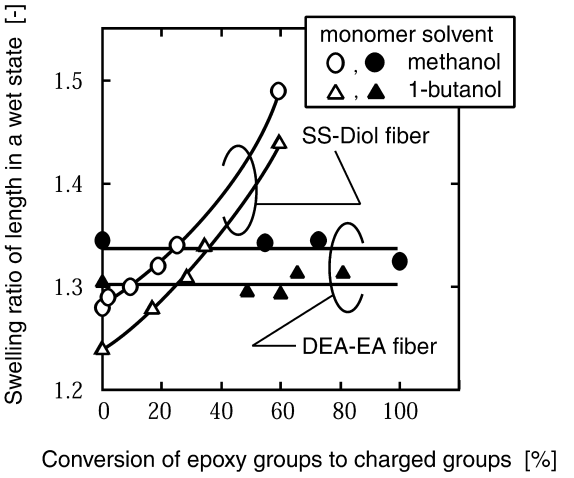


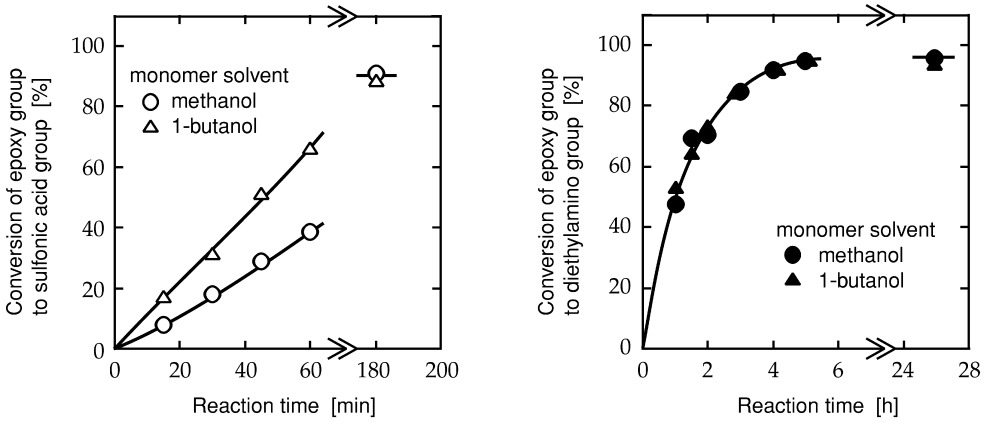
Fig. 6. Swelling ratio in length.

amino groups are shown in Fig. 5(a) and (b), respectively. For both additions, final conversions agreed well for the GMA-grafted fibers prepared with methanol and 1-butanol as a solvent.

3.2. Membrane properties

The swelling ratio, defined by the ratio in a wet state of the length of the SS-Diol (M or B, 190, *x*) fiber or the DEA-EA (M or B, 190, *x*) fiber to the length of the trunk fiber, is shown in Fig. 6 as a function of the conversion. The dimensions of the SS-Diol fibers increased with increasing conversion, whereas those of the DEA-EA fibers were almost constant irrespective of the conversion. For the introduction of the charged groups, the GMA-grafted fiber prepared with methanol as a solvent swelled highly compared to that prepared with 1-butanol.

The SS-Diol (M or B, 190, 60) and DEA-EA (M or B, 190, 100) fibers were analyzed by the micro-FT-IR spectra. Peaks at 2920, 1160 and 1150 cm^{-1}



(a) -SO_3H; SS-Diol fiber (b) -$N(C_2H_5)_2$; DEA-EA fiber

Fig. 5. Conversion of the epoxy groups to charged groups.

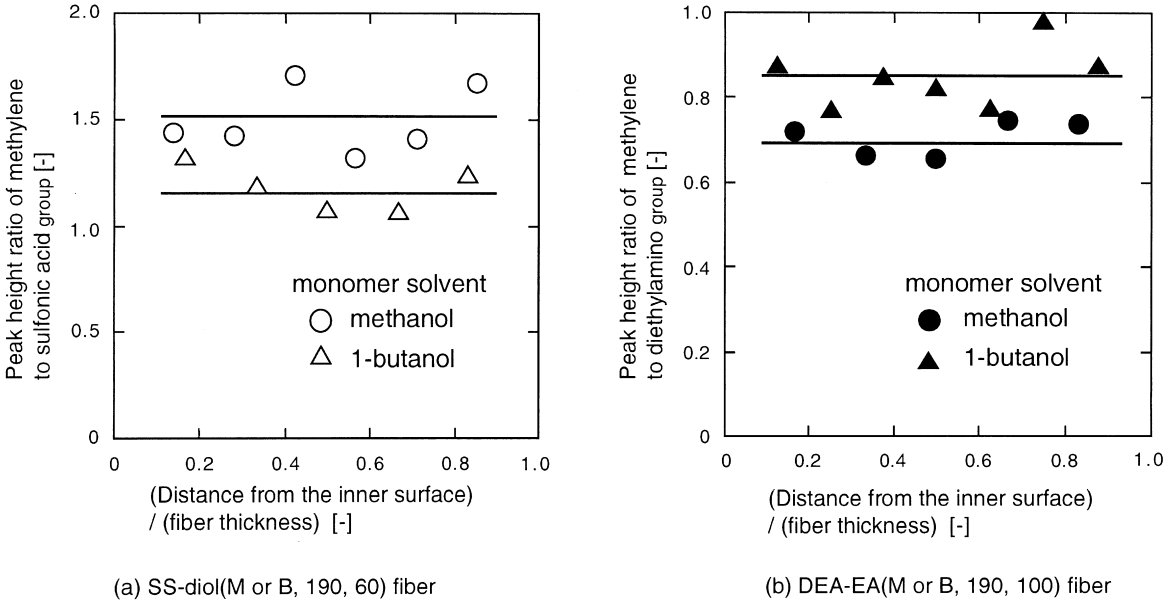


Fig. 7. Distribution of the charged groups across the SS-Diol and DEA-EA fibers.

are characteristic peaks of methylene, sulfonic acid group, and diethylamino group, respectively. Profiles of peak height ratio of methylene to sulfonic acid and diethylamino groups across the fiber thickness are shown in Fig. 7(a) and (b), respectively. Uniform profiles indicate that both poly-GMA chain and charged groups were uniformly appended across the SS-Diol (M or B, 190, 60) and DEA-EA (M or B, 190, 100) fibers.

3.3. Degree of protein multilayer binding to charged polymer brushes

Protein binding to the charged polymer brushes

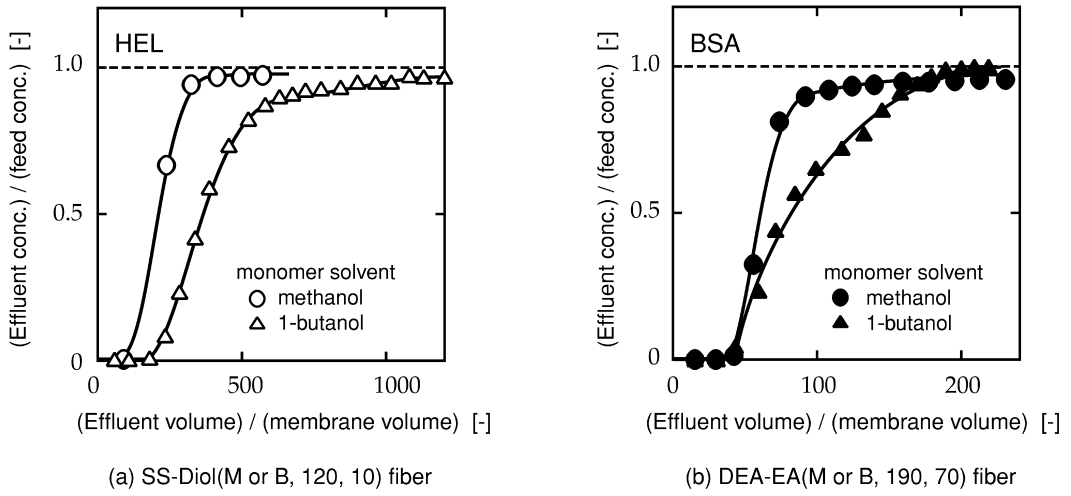


Fig. 8. Breakthrough curves of the SS-Diol fiber for HEL and the DEA-EA fiber for BSA.

Table 1
Comparison of degree of protein multilayer binding

	SS-Diol fiber		DEA-EA fiber	
	Methanol	1-Butanol	Methanol	1-Butanol
Dg (%)	120		190	
Conv. (%)	10	11	70	65
EBC[a] (mg/g)	180	380	250	370
Degree of multilayer binding[b] (−)	15	32	4.3	6.4
Flux of protein solution[c] (m/h)				
Initial	0.36	0.058	0.29	0.023
Final	0.55	0.018	0.12	0.0078

[a] Equilibrium binding capacity.
[b] HEL for SS-Diol fiber, BSA for DEA-EA fiber.
[c] Permeation pressure, 0.1 MPa; temperature, 298 K.

grafted onto the porous hollow-fiber membranes was evaluated in a permeation mode [13]. Breakthrough curves, i.e. protein concentration changes in the effluent as a function of effluent volume, are shown in Fig. 8(a) and (b) for the binding of HEL to the SS-Diol (M or B, 120, 10) fiber and BSA to the DEA-EA (M or B, 190, 70) fiber, respectively. In both figures, the EBC of the charged polymer brush originating from the poly-GMA brush prepared with 1-butanol was higher than that originating from the poly-GMA brush with methanol, as listed in Table 1: the EBC of HEL for the SS-Diol (B, 120, 11) fiber of 380 mg/g was 2.1-fold of that for the SS-Diol (M, 120, 10) fiber of 180 mg/g, the EBC of BSA for the DEA-EA (B, 190, 65) fiber of 370 mg/g was 1.5-fold of that for the DEA-EA (M, 190, 70) fiber of 250 mg/g.

The liquid permeability, i.e. the flux defined by dividing the permeation rate of the protein solution by the inside surface area of the fiber before and after the protein binding to the charged polymer brush, is also shown in Table 1. Both initial and final fluxes for the SS-Diol and DEA-EA fibers prepared with 1-butanol were much lower than those prepared with methanol.

4. Discussion

We employed the preirradiation technique for grafting glycidyl methacrylate (GMA) onto the porous hollow-fiber membrane because of the negligible amount of GMA homopolymer. An electron beam was irradiated throughout the entire membrane to form radicals. From a micro-FT-IR measurement, the poly-GMA chain was found to be appended uniformly across the membrane. Two categories of formation site of the graft chain are recognized: those inside the polyethylene matrix and those on the pore surface. The former polymer chains allow the matrix to swell, whereas the introduction of charged groups into the latter graft chain, i.e. the polymer brush, induces expansion to capture the proteins in multilayers. The effect of the solvent for GMA on grafting on the polymer brush length is discussed in terms of swelling, protein adsorptivity, and liquid permeability.

The SS-Diol (M, 190, *x*) and DEA-EA (M, 190, *x*) fibers swelled more highly in water than the SS-Diol (B, 190, *x*) and DEA-EA (B, 190, *x*) fibers. This indicates that the portion of the poly-GMA chain in the polyethylene matrix is higher for the same degree of GMA grafting in methanol than in 1-butanol.

Permeabilities of the SS-Diol (B, 120, 11) and DEA-EA (B, 190, 65) fibers for buffer solutions were much lower than those of the SS-Diol (M, 120, 10) and DEA-EA (M, 190, 70) fibers. This demonstrates that a longer polymer brush is produced in 1-butanol compared to that in methanol.

The SS-Diol (B, 120, 11) and DEA-EA (B, 190, 65) fibers exhibited 2.1- and 1.5-fold higher EBC for hen egg lysozyme (HEL) and bovine serum albumin (BSA) than the SS-Diol (M, 120, 10) and DEA-EA (M, 190, 70) fibers, respectively. This fact shows that a longer polymer brush on the SS-Diol (B, 120, 11) and DEA-EA (B, 190, 65) fibers extends from

the pore surface toward the pore interior to capture HEL and BSA, respectively, at a higher degree of multilayer binding.

The elution percentages of HEL and BSA that adsorbed onto the polymer brush with 0.50 *M* NaCl, defined by Eq. (6), were both 100%; nonselective adsorption of the proteins onto the polymer brush was negligible. The quantitative elution revealed that the polyethylene surface is covered by the hydrophilic graft chain [9]. This enables a stable repetition of adsorption and elution of the proteins.

As for the radiation-induced graft polymerization, the length of the polymer brush grafted onto the pore surface of the porous hollow-fiber membrane is governed by various factors: radical concentration [14,15], penetration depth of solvent into the trunk polymer matrix [16–18], and growth, chain-transfer and termination rates of the graft polymerization [19–21]. In this study, the two solvents, methanol and 1-butanol, for GMA were compared for their influence on the polymer brush length. Comparison of swelling, protein adsorptivity, and liquid permeability of the resultant membranes indicates that 1-butanol provide a longer polymer brush on the pore surface of the porous polyethylene hollow-fiber membrane than does methanol.

At present, we cannot characterize the polymer brush based on the determination of molecular mass distribution because of experimental difficulty in isolating the polymer brush from the graft chain in the matrix. Here we suggested a novel characterization of the polymer brush based on performance involving the polymer brush.

5. Conclusion

An electron beam was irradiated onto porous membranes made of polyethylene to produce radicals as a starting site for the graft polymerization of GMA. Subsequently, by the immersion in GMA diluted with methanol or 1-butanol as a solvent, the polymer chain was appended uniformly throughout its membrane thickness. The graft chain is classified according to its formation site: the graft chain embedded in the polyethylene matrix, and the polymer chain extending from the pore surface toward the pore interior, i.e. the polymer brush. Two charged groups, i.e. sulfonic acid and diethylamino groups, were introduced into the resultant poly-GMA brush to capture two proteins, hen egg lysozyme and bovine serum albumin, respectively, in a permeation mode. From the change in liquid permeability and degree of protein multilayer binding caused by the presence of the polymer brush, it was demonstrated that a longer polymer brush was prepared in 1-butanol than in methanol.

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References

[1] S. Sugiyama, S. Tsuneda, K. Saito, S. Furusaki, T. Sugo, K. Makuuchi, React. Polym. 21 (1993) 187.
[2] H. Yamagishi, K. Saito, S. Furusaki, T. Sugo, I. Ishigaki, Ind. Eng. Chem. Res. 30 (1991) 2234.
[3] M. Kim, M. Sasaki, K. Saito, K. Sugita, T. Sugo, Biotechnol. Prog. 14 (1998) 661.
[4] W. Muller, J. Chromatogr. 510 (1990) 133.
[5] S. Tsuneda, K. Saito, S. Furusaki, T. Sugo, J. Chromatogr. A 689 (1995) 211.
[6] T. Mizota, S. Tsuneda, K. Saito, T. Sugo, Ind. Eng. Chem. Res. 33 (1994) 2215.
[7] H. Shinano, S. Tsuneda, K. Saito, S. Furusaki, T. Sugo, Biotechnol. Prog. 9 (1993) 193.
[8] T. Kawai, K. Sugita, K. Saito, T. Sugo, Macromolecules 33 (2000) 1306.
[9] M. Kim, J. Kojima, K. Saito, S. Furusaki, T. Sugo, Biotechnol. Prog. 10 (1994) 114.
[10] N. Sasagawa, K. Saito, K. Sugita, S. Kunori, T. Sugo, J. Chromatogr. A 848 (1999) 161.
[11] S. Tsuneda, H. Shinano, K. Saito, S. Furusaki, T. Sugo, Biotechnol. Prog. 10 (1994) 76.
[12] I. Koguma, K. Sugita, K. Saito, T. Sugo, Biotechnol. Prog. 16 (2000) 456.
[13] S. Matoba, S. Tsuneda, K. Saito, S. Furusaki, T. Sugo, Bio/Technology 13 (1995) 795.
[14] K. Uezu, K. Saito, S. Furusaki, T. Sugo, I. Ishigaki, Radiat. Phys. Chem. 40 (1992) 31.
[15] W. Lee, S. Furusaki, J. Kanno, K. Saito, T. Sugo, Chem. Mater. 11 (1999) 3091.

[16] K. Hayakawa, C. Lin, K. Kawase, T. Matsuda, Kobunshi Kagaku 20 (1963) 540.
[17] V.D. Athale, S.C. Rathi, React. Polym. 34 (1997) 11.
[18] E.A. Hegazy, H.A. El-Rethim, N.A. Khalifa, A.E. Ali, Radiat. Phys. Chem. 55 (1999) 219.
[19] K. Hayakawa, K. Kawase, T. Matsuda, Kobunshi Kagaku 20 (1963) 609.
[20] S. Watabe, I. Ito, Kogyo Kagaku Zasshi 66 (1963) 850.
[21] T. Yumoto, T. Matsuda, Nippon Kagaku Kaishi (1972) 369.