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CHARGED POLYMER BRUSH GRAFTED ONTO POROUS HOLLOW-FIBER MEMBRANE IMPROVES SEPARATION AND REACTION IN BIOTECHNOLOGY

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ABSTRACT

An epoxy-group-containing polymer brush anchored onto the pore surface of a porous hollow-fiber membrane, followed by the introduction of ionizable groups, extends due to mutual electrostatic repulsion, and holds proteins in multilayers. This phenomenon provides novel insight into the static structure and dynamic behavior of the charged polymer brush, and enables highly efficient protein recovery, chiral separation, and enzymatic reaction. Various proteins were recovered using the porous hollow-fiber membranes immobilizing the charged polymer brush at a higher rate and higher capacity compared to bead-packed column because of convection of the protein solution through the pores and multilayering of the proteins in the polymer brush.

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INTRODUCTION

A polymer brush is defined as long polymer chains grafted with sufficiently high density onto the surface of polymeric and inorganic substrates. The interface formed by the polymer brush has attracted considerable attention from the viewpoints of adsorption, adhesion, lubrication, and wetting (1,2). Most theoretical (3,4) and experimental (5–9) studies have addressed a neutral, i.e., uncharged, polymer brush to elucidate the conformation and dynamics of such polymer brushes. Zhulina and co-workers (10,11) theoretically studied a charged polymer brush, i.e., polyelectrolyte brush, immersed in various solutions. However, experimental observations of the charged polymer brush are required in order to understand the behavior well.

Radiation-induced graft polymerization is a powerful method to anchor the polymer brush onto polymeric substrates of various shapes and quantities (12). The polymer brush on the pore surface of a porous hollow-fiber membrane will enable high-performance separation and reaction (Fig. 1). First, an epoxy-group-containing polymer brush, i.e., poly-glycidyl methacrylate (poly-GMA) brush, was polymerized onto the porous hollow-fiber membrane by the radiation-induced graft polymerization, and then the resultant epoxy group of the polymer brush was converted into various functional groups capable of efficiently collecting proteins (13) and removing metal ions (14).

Immobilization of the polymer brush on porous flat-sheet membranes has been reviewed by Ito (15), where the polymer brush was formed in the neighborhood of the membrane surfaces by the plasma-induced graft polymerization. The resultant membranes are expected to be mainly applicable to stimuli-sensitive materials.

In this article, the epoxy group of the poly-GMA brush anchored onto the porous hollow-fiber membrane by radiation-induced graft polymerization, was converted to two adjacent hydroxyl (diol) groups and ionizable groups. In particular, the ionizable-group-containing polymer brush (charged polymer brush) exhibits a characteristic static structure and dynamic behavior. From the determinations of liquid permeability and protein adsorptivity of the porous hollow-fiber membrane, the effective length of the charged polymer brush anchored to the pore surface was discussed, and the applicability of protein multilayering induced by the charged polymer brush to biotechnology was demonstrated.

IMMOBILIZATION OF POLYMER BRUSH

Radical formation on a polymeric substrate by the electron beam, gamma ray, photo, and plasma irradiation is required to immobilize the

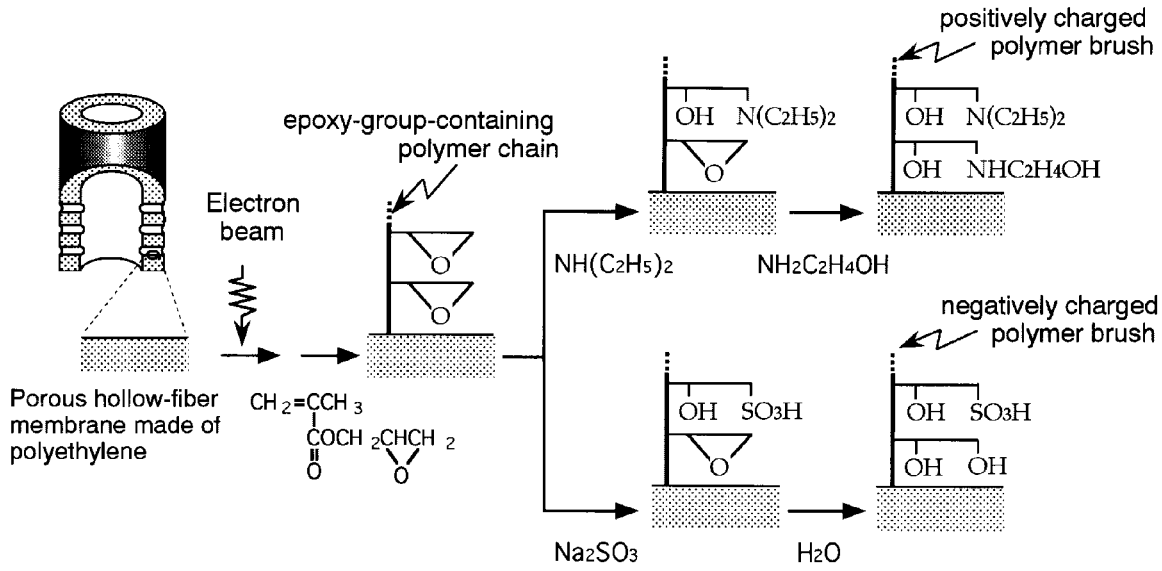

Figure 1. Immobilization of functional polymer brush by radiation-induced graft polymerization and subsequent chemical modification. For example, an epoxy-group-containing polymer chain was anchored onto the pore surface of an electron-beam-irradiated porous hollow-fiber membrane. Subsequently, the resultant epoxy group was converted to an ionizable group such as sulfonic acid and diethylamino groups. After graft polymerization at a degree of grafting of 200%, the porous structure of the membrane was found to be retained based on a scanning electron microscopic observation.

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polymer brush. Of these, radiation-induced graft polymerization is advantageous in that substrates of various shapes can be modified on both laboratory and commercial scales. According to the irradiation technique, radiation-induced graft polymerization is classified into pre-irradiation and simultaneous irradiation techniques. The former technique has practical merits of smaller amounts of homopolymers, and segregation of the polymerization process from irradiation.

A porous hollow-fiber membrane made of polyethylene (Asahi Kasei Corporation, Japan) was used as a substrate. This membrane was used for microfiltration of the colloidal suspension and microbial broth. The hollow fiber had inner and outer diameters of 2 and 3 mm, respectively, with a pore diameter of about 500 nm and a porosity of 70%. Radiation-induced graft polymerization was suitable for producing radicals throughout the porous hollow-fiber membrane, resulting in the uniform immobilization of the polymer brush across the membrane thickness.

The reasons for selecting the porous hollow-fiber membrane as the substrate are as follows:

1. The reduction in pore diameter is accompanied by the immobilization of the polymer brush on the pore surface. The determination of permeability, defined as flux evaluated by dividing the flow rate at a constant permeation pressure by the inside surface area of the hollow-fiber membrane, enables the estimation of the effective height of the polymer brush based on hydrodynamic analysis (16). Then, water breaking pressure, defined as the minimum pressure required to initiate water permeation through a dried porous hollow-fiber membrane, depends on the degree of hydrophilicity of the polymer brush anchored on the pore surface (17). Since, at present, a direct characterization, e.g., the determination of molecular weight distribution of the polymer brush isolated from the substrate, is difficult, a simple characterization of the polymer brush by the determination of the liquid permeability of the porous membrane immobilizing the polymer brush is effective.
2. A protein solution is forced to permeate outward through the pores surrounded by a functional polymer brush. The diffusional mass-transfer path of the protein in the solution to the functional group can be minimized because the protein can be transported by the permeation flow driven by the transmembrane pressure (18). Therefore, an adsorptive method using porous membranes, which immobilize the polymer brush is superior to the conventional method using a bead-packed bed (19), where the overall adsorption rate is governed by the diffusion of the protein into the bead interior. Moreover, a porous membrane of hollow-fiber form is more advantageous than that of flat-

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sheet form because a higher ratio of surface area to volume is attained by fabricating the porous hollow-fiber membrane module for scale-up of protein recovery.

In the pre-irradiation technique, first, radicals are formed on the substrate by the irradiation of electron beam in a nitrogen atmosphere at ambient temperature. The electron beam was irradiated using a cascade-type accelerator (Dynamitron model IEA 3000-25-2, Radiation Dynamics, Inc., New York). Secondly, the irradiated substrate is immersed in a monomer solution previously deaerated to initiate the graft polymerization. Here, the alkyl radical contributing to the grafting was found not to decay below the glass transition temperature of the substrate (20). This is practically favorable in that the two procedures of irradiation and grafting can be segregated; the polymer brush is easily appended onto various substrates apart from irradiation facilities.

A uniform immobilization of the polymer brush was attainable across the porous hollow-fiber membrane thickness of about 0.5 mm. The degree of grafting is defined as the percentage weight increase of the substrate due to the polymer brush:

$$\text{degree of grafting} = 100(W_1 - W_0)/W_0,$$

where W_0 and W_1 are the weights of the substrate and the polymer brush-grafted substrate, respectively. The poly-GMA brush was anchored at a degree of grafting ranging of up to 200%, which was followed by a reaction with diethylamine. The HCl-adsorbed polymer brush containing a diethylamino group exhibited a uniform chloride profile across the membrane thickness as observed by the x-ray microanalysis (21); this demonstrates that the polymer brush was anchored uniformly throughout the membrane by the radiation-induced graft polymerization. This bulky modification is favorable for high-capacity adsorptive separations.

The poly-GMA brush can be converted to a functional polymer brush at a definite conversion under the control of solvent, reaction temperature, and time. For example, a molar conversion, defined by the molar percentage of the ionizable group introduced to the epoxy group before reaction, ranged up to 90% at a degree of grafting of 100%. The functional group density of the resultant porous hollow-fiber membrane exceeded that of a commercially available ion-exchange bead.

CHARACTERIZATION OF POLYMER BRUSH INTERFACE

Over a threshold amount, the polymer brush will hinder the substrate pore surface; conversion of the poly-GMA brush into the diol-group-

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containing polymer brush (diol polymer brush) by the addition of water to the epoxy ring will render the pore surface hydrophilic. Pure water flux of the dried membrane is determined after a methanol treatment, i.e., immersion in methanol and then in water. When the diol polymer brush of about 7 mol kg^{-1} of the polyethylene substrate was anchored, the pure water flux of the membrane without methanol treatment coincided with that of the membrane with methanol treatment (Fig. 2a) (22). This observation suggests that an increasing amount of the diol polymer brush anchored on the pore surface produced a more self-wettable interface.

Evaluation of non-selective adsorption of proteins is a way to measure the interfacial characteristics. The diol polymer brush reduced the amount of bovine γ-globulin (BGG) adsorbed non-selectively (Fig. 2b). At an excess of about 7 mol of the diol polymer brush per kg of the polyethylene substrate, the equilibrium binding capacity for BGG leveled off because the substrate was sufficiently covered by the diol polymer brush to minimize the non-selective adsorption via hydrophobic interaction, i.e., to hydrophilize the pore surface. The dependence of the BGG equilibrium binding capacity on the diol group density agreed well with that of pure water flux.

Iwata et al. (18) introduced a diol group into the polymer brush via a ring-opening reaction of the epoxy group to reduce unspecific adsorption of undesired proteins onto the membrane. The amount of bovine serum albumin, in units of grams per liter, onto the diol-containing porous hollow-fiber membrane was as low as that of bovine serum albumin to agarose, where the value of the density of the modified hollow-fiber membrane, evaluated by dividing the weight of dry membrane by the volume, excluding the lumen part, of wet membrane, was approximately 0.4 g-dry mL^{-1}-wet for the diol-group-containing porous hollow-fiber membrane. The diol group was selected as a coexisting group of cation-exchange and hydrophobic groups on the polymer brush.

Introduction of an ionizable group, i.e., ion-exchange group, to the polymer brush causes the polymer brush to stretch from the pore surface toward the pore interior due to mutual electrostatic repulsion (Fig. 3a). The degree of extension of the polymer brush is controllable by the molar conversion of the epoxy group to the ionizable group (23). A protein solution was forced to permeate through the pores immobilizing the charged polymer brush. For example, bovine serum albumin (BSA; $M_r = 68,000$, pI 5.0) dissolved in a buffer solution was bound to a diethylamino-group-containing polymer brush via electrostatic interaction during permeation. The amount of BSA captured by the polymer brush, determined by integrating the difference in BSA concentration between the feed and effluent, was converted to the degree of multilayer binding of protein by dividing it by a theoretical value for

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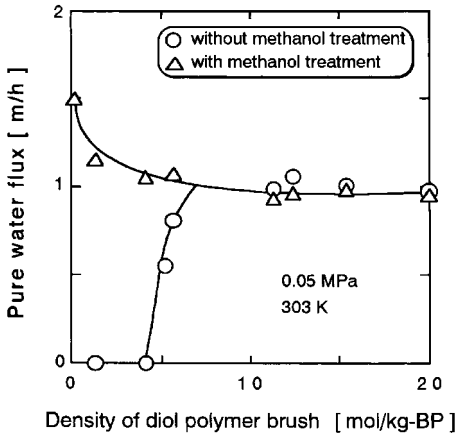
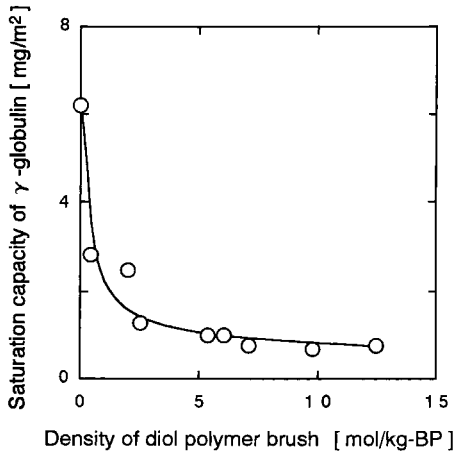


(a) Liquid Permeability



(b) Protein adsorptivity

Figure 2. Hydrophilization of a polyethylene pore surface by diol-group-containing polymer brush. An increase in the density of the diol polymer brush rendered the surface self-wettable, resulting in the reduction of the non-selective adsorption of proteins.

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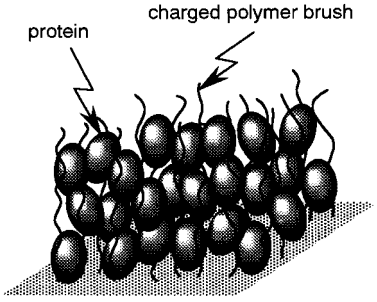
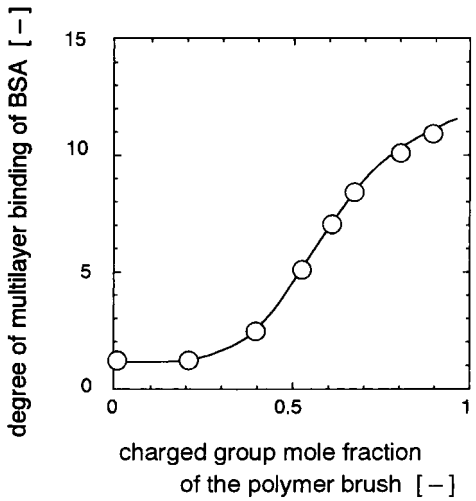


(a)



(b)

Figure 3. Protein multilayering by the charged polymer brush. Driven by a pressure difference across the membrane, a protein solution was permeated through the pores of the porous hollow-fiber membrane that immobilizes the charged polymer brush. The integration of the protein concentration difference between the feed and effluent gives the amount of protein adsorbed. The degree of multilayer binding of the protein was evaluated by dividing the adsorbed amount by a theoretical value for monolayer binding capacity. An increasing molar conversion achieved a higher degree of multilayer binding of the protein because mutual repulsion of the polymer brush forms a three-dimensional space for binding the proteins.

the monolayer binding capacity of protein:

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

$$\text{monolayer binding capacity} = a_v M_r/(aN_A),$$

where a_v, a, M_r, and N_A are the specific surface area of the hollow fiber, the area occupied by a protein molecule, the molecular mass of the protein, and Avogadro's number, respectively. With an increase of mole fraction in the diethylamino group of the polymer brush, the degree of multilayer binding of BSA increased, and reached 11 at a molar conversion of 80% (Fig. 3b).

Two ionizable groups, i.e., sulfonic-acid and diethylamino groups, were introduced to the poly-GMA brush anchored on the pore surface of the porous hollow-fiber membrane (24). Aminoacylase ($M_r = 86,000$) dissolved in an appropriate buffer was forced to permeate through the pores to bind the respective polymer brush. Changes in both protein concentration and permeation pressure under a constant flow rate of the protein solution were continuously determined: binding of aminoacylase to the sulfonic-acid polymer brush induced a decrease in permeation pressure, whereas that to the diethylamino polymer brush induced an increase in permeation pressure. This result occurs when there is a difference in the initial extension structure of the polymer brush: the sulfonic-acid polymer brush stretches higher because of higher ionization, resulting in the entanglement of the protein to form a compressed structure.

Proteins captured by the polymer brush in multilayers were quantitatively eluted by permeating 0.5 *M* NaCl. During elution, the permeation pressure decreased because the polymer brush collapsed while releasing the adsorbed protein.

The charged polymer brush immobilized onto the pore surface of the porous hollow-fiber membrane can hold proteins in multilayers, e.g., 11 layers of BSA corresponding to about 13 nm in an end-on orientation. This is an effective height for the polymer brush for protein recovery. Thus, experimental determination of liquid permeability and protein adsorptivity provides quantitative image of the polymer brush anchored on the pore surface.

MULTILAYERING OF PROTEINS BY POLYMER BRUSH

At present, protein purification is mainly achieved by chromatography using a bead-packed column. Proteins bind to the functional groups attached to the bead matrix such as agarose and polystyrene based on electrostatic, hydrophobic, and affinity interactions. However, the column chromatography

requires a long time and tedious procedures for protein processing. To overcome this drawback, a novel method using a porous membrane to immobilize the charged polymer brush was suggested. This method enabled high-rate and high-capacity adsorption of proteins because of the convective flow of protein solutions across the porous membrane and the multilayer binding of proteins into the charged polymer brush.

Lysozyme ($M_r = 14,300$, pI 11) contained in egg-white at a concentration of about 3 wt% decomposes the microbial membrane wall; therefore, lysozyme has been used as a component of cold medicines. Highly efficient adsorption of lysozyme was verified using the charged polymer brush (25,26). Lysozyme dissolved in a buffer (pH 8) was permeated outward across the porous hollow-fiber membrane immobilizing the sulfonic-acid polymer brush (dg 100%, molar conversion 35%). Prior to lysozyme permeation, the permeation of $MgCl_2$ solution was effectively achieved because Mg ions ionically crosslink the polymer brush to significantly improve the liquid permeability (26). The protein concentration change in the effluent as a function of the effluent volume, i.e., breakthrough curve, is shown in Fig. 4. Initially, lysozyme did not appear in the effluent penetrating through the outside surface of the porous hollow-fiber membrane; however, with increasing effluent volume, the lysozyme concentration gradually increased. Finally, the concentration of the effluent reached that of the feed. The equilibrium binding capacity of the membrane for lysozyme was calculated as $0.42\,g\,g^{-1}$ of the membrane, which amounted to a degree of multilayer binding of 38. After equilibration of the protein adsorption, washing of the pores and elution of the adsorbed protein were performed during the permeation of the buffer and 0.5 *M* NaCl, respectively. The lysozyme bound to the polymer brush was quantitatively eluted to yield a highly-concentrated protein solution (26).

Various proteins such as albumin (27), urease (23), lactoglobulin (23), and aminoacylase (24) were captured by the diethylamino polymer brush on the pore surface at a high rate and high capacity. The high rate is ascribed to the negligible diffusional mass-transfer resistance of the protein in the solution to the ionizable group of the polymer chain aided by the permeation flow through the pores of the porous hollow-fiber membranes. The high capacity is explained by the three-dimensional, i.e., multilayer, binding of the proteins into the charged polymer chain. Membrane chromatography was suggested in 1988 by Brandt et al. (28) to overcome a dilemma in column chromatography between smaller diffusional mass-transfer resistance into smaller beads and higher pressure loss across the bed charged with smaller beads. In addition, tentacle beads were prepared in 1990 by Muller (29) to raise the protein binding capacity by grafting the ion-exchange polymer chain onto the outer surface of a polymeric bead. Here, the radiation-induced graft polymerization of the GMA on the porous membrane and subsequent chemical modification of the epoxy group to the ionizable group

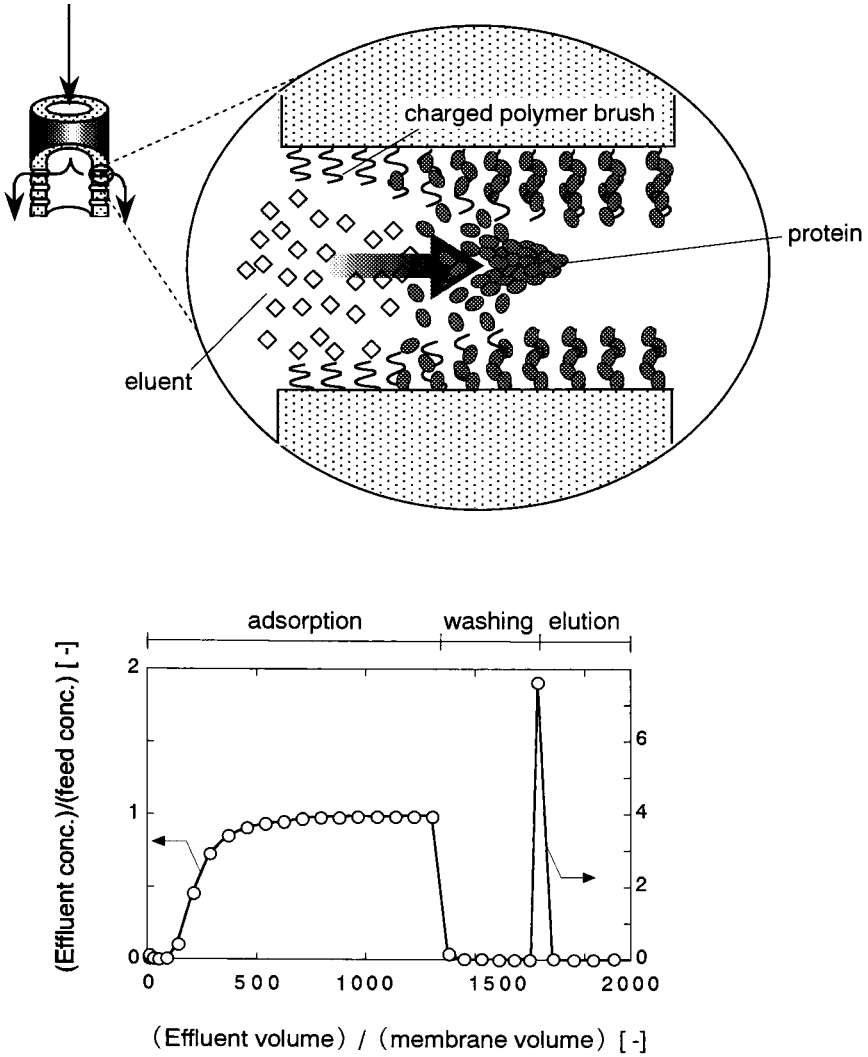


Figure 4. High-rate and high-capacity binding of proteins by the charged polymer brush. Lysozyme was bound to the charged polymer brush in multilayers, resulting in 45-fold higher capacity of the theoretical monolayer binding capacity. Lysozyme was transported by convective flow through the pores to minimize the diffusional mass-transfer path of the protein to the polymer brush, resulting in a high-rate protein binding. The lysozyme bound to the polymer brush was quantitatively released to yield a highly-concentrated protein solution.

simultaneously realized two concepts of permeation flow through the pores and tentacle binding along the pore surface.

The charged polymer brush can bind proteins selectively (30). The ion-exchange group introduced to the polymer brush exhibits different electrostatic interactions with proteins according to the protein surface charge density, size, and shape. A mixture of ovotransferrin (pI 6.1) and ovomucoid (pI 4.1) in a buffer (pH 8.0) was fed to the inside surface of the porous hollow-fiber membrane, the pores of which were surrounded by the diethylamino-group-containing polymer brush. The protein concentration changes in the effluent revealed that the protein with a stronger affinity for the polymer brush, ovomucoid, expelled the protein with a weaker affinity previously captured by the polymer brush, ovotransferrin, with increasing feed volume (Fig. 5). Irrespective of a shorter residence time of the order of 10 sec across the membrane thickness, a phenomenon similar to the bead-packed column with a longer residence time of the order of 500 sec across the column was observed.

Bovine serum albumin (BSA) has been reported to have a recognition site for L-compounds of enantiomers (31). Enrichment of the L-compounds is being

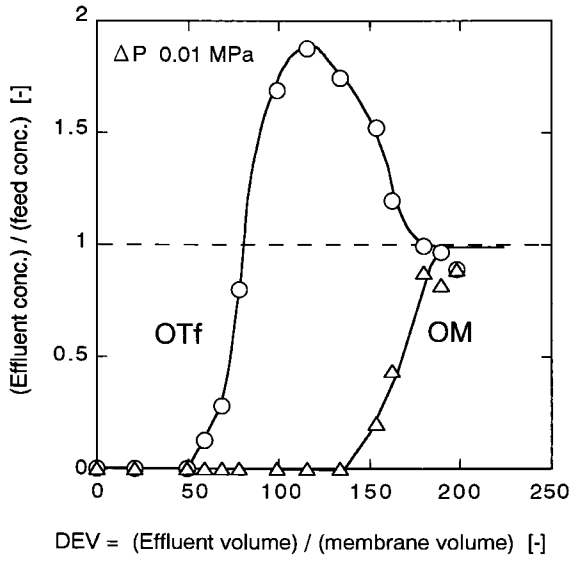


Figure 5. Breakthrough curves of a binary protein system. A mixture of ovomucoid (OM) and ovotransferrin (OTf) was forced to permeate through the pores of the membrane immobilizing the anion-exchange polymer brush. A roll-up peak is observed; the stronger affinity protein OM displaces the weaker affinity protein OTf, leading to a rise in the effluent concentration of OTf above the feed value.

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demanded in order to commercialize safe pharmaceuticals. Bovine serum albumin multilayered by the polymer chain anchored on the pore surface of a porous hollow-fiber membrane worked efficiently as a chiral selector, as compared to BSA adsorbed on conventional beads in monolayer. The degree of multilayering of BSA of the membrane was changed between one and six by varying the molar conversion of the epoxy group to the diethylamino group in the range of 40–90%. A buffer as a mobile phase was forced to permeate outward from the inside to the outside through the pores, and subsequently a constant amount of DL-tryptophan was injected as an analyte to the mobile phase. From a chromatogram obtainable by the permeation (Fig. 6 as an example), the separation factor defined as the ratio of the retention time of L-tryptophan to that of D-tryptophan was found to increase with an increase in the degree of multilayer binding of BSA in the diethylamino polymer brush (32,33) (Fig. 7). In addition, the separation factor was constant irrespective of the flow rate of the mobile phase because of the negligible diffusional mass-transfer resistance of the analyte to the chiral selector aided by the convective flow through the pores (33). Other combinations of chiral selector and enantiomer were effective for chiral separations.

Multilayer binding of enzymes as a protein on the porous surface is expected to bring about a higher activity than the entrapment of enzymes in polymeric gels. For example, aminoacylase capable of asymmetrical hydrolysis,

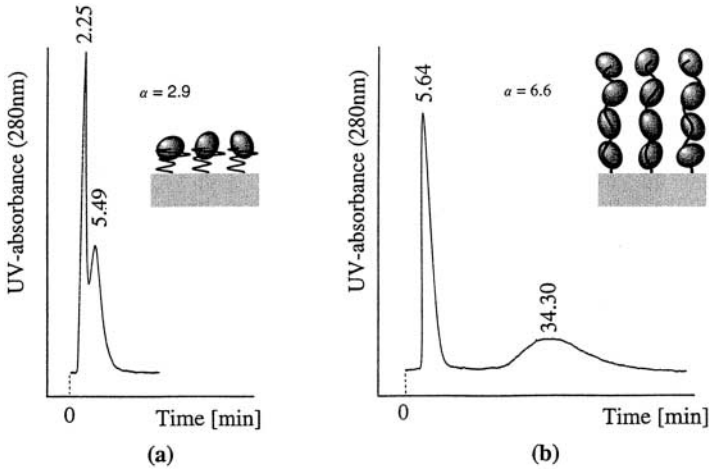

Figure 6. Chiral separation of DL-tryptophan during the permeation of a racemic solution through a BSA-multilayered porous hollow-fiber membrane. (a) Degree of multilayer binding: monolayer. (b) Degree of multilayer binding: four layers.

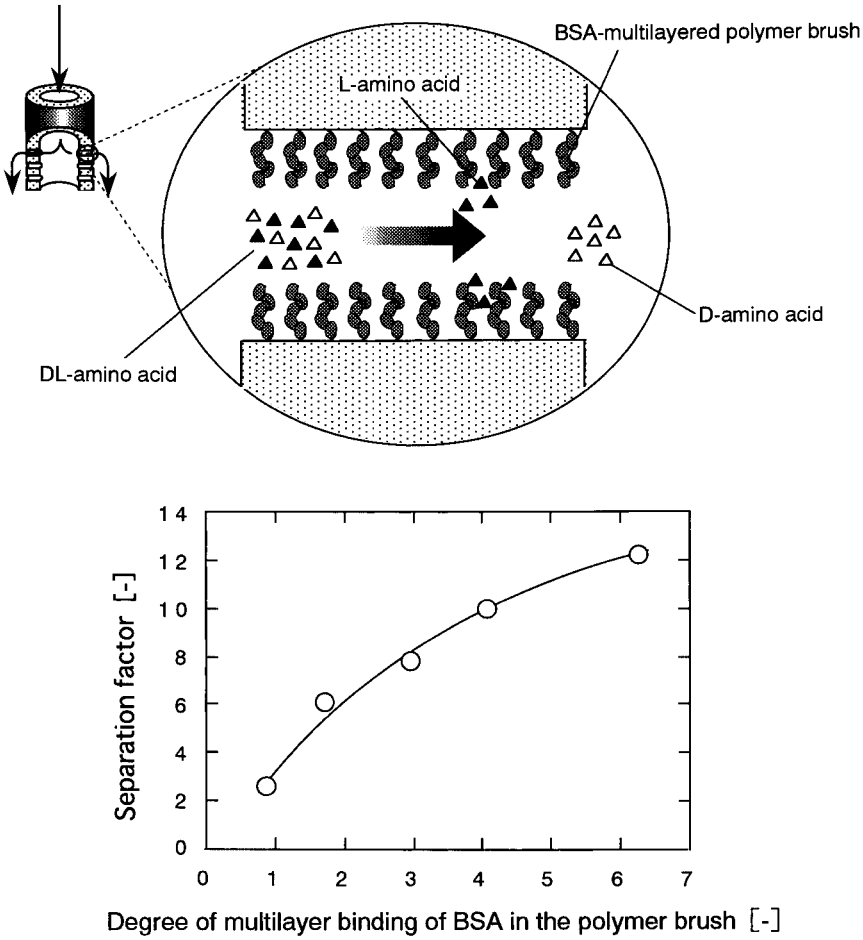

Figure 7. High resolution of D-L-tryptophan by bovine serum albumin-multilayered polymer brush. Bovine serum albumin (BSA) as a chiral selector was multilayered by the charged polymer brush onto the pore surface of the porous hollow-fiber membrane. Then, DL-tryptophan as an analyte was injected into the permeation flow of a mobile phase through the pores. A higher degree of multilayer binding of BSA produced a larger difference in the retention times of DL-tryptophan, i.e., a higher separation factor.

dissolved in a buffer, was forced to permeate through the pores immobilizing the diethylamino polymer brush to yield a degree of multilayer binding of about 15. Subsequent crosslinking of aminoacylase with glutaraldehyde in the polymer brush prevented enzyme leakage from the polymer brush. Permeation of acetyl-

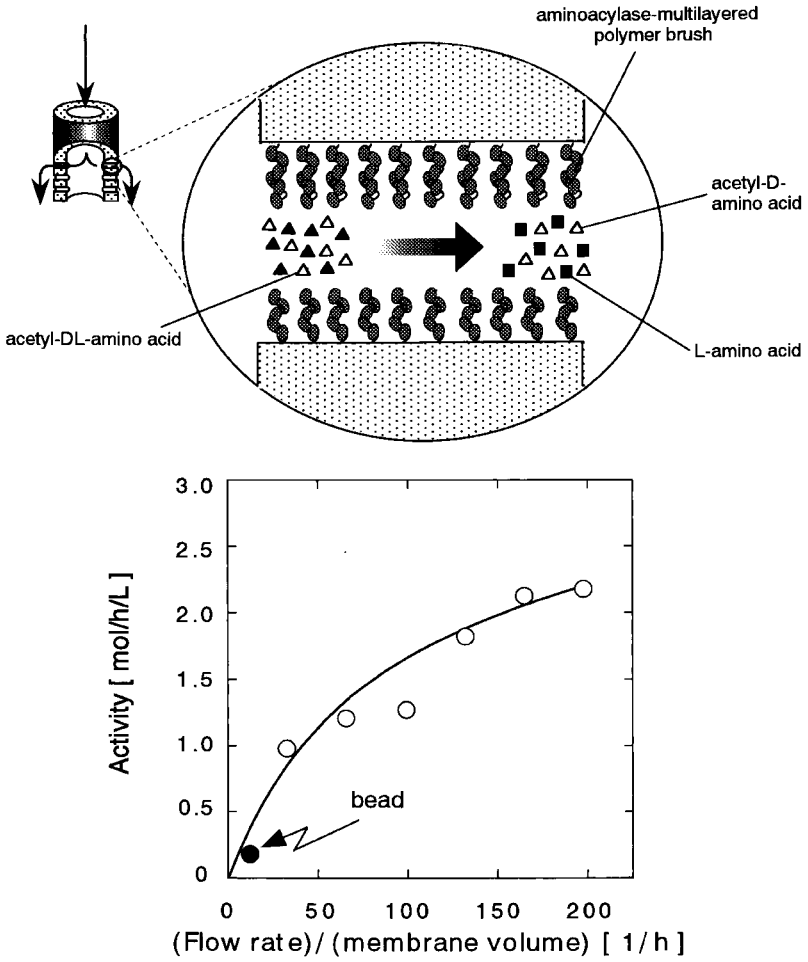

Figure 8. High activity of asymmetrical hydrolysis of acetyl-DL-methionine by aminoacylase-multilayered polymer brush. First, aminoacylase was captured by the charged polymer brush at a degree of multilayer binding of six. Second, the enzymes were crosslinked with glutaraldehyde to prevent the leakage from the polymer brush. Third, acetyl-DL-methionine as a substrate was forced to permeate through the pores immobilizing the aminoacylase-multilayered polymer brush. During the permeation of the substrate solution, acetyl-L-methionine was specifically hydrolyzed to liberate L-methionine. At different flow rates of the substrate solution, enzymatic activity was evaluated by dividing the resultant L-methionine concentration times flow rate by the membrane volume, including the lumen part. Due to the enzyme multilayering, the enzyme exhibited a higher activity even at a higher flow rate, compared to the enzyme immobilized on a glass bead. The activity for an aminoacylase-immobilized glass bead (35) is denoted by a solid circle in the figure.

DL-methionine solution through the pores surrounded by the aminoacylase-multilayered polymer brush enabled an activity of $4.1\,mol\,L^{-1}\,hr^{-1}$ even at a space velocity of $200\,hr^{-1}$ (Fig. 8) (34), i.e., residence time of 1 sec, which was fourfold higher than the activity of the aminoacylase-immobilized bead (35) at a lower space velocity of $10\,hr^{-1}$. The polymer brush capable of multilayering enzymes provides a novel matrix for immobilization with high capacity (36,37).

Grafting and subsequent chemical modifications induced swelling of the porous hollow-fiber membranes because some of the polymer chains formed deep in the polyethylene matrix; however, the module was fabricated in the same manner as the original hollow-fiber membranes used for microfiltration (38).

Practical applications of modified porous hollow-fiber membranes will be limited by sterilization because of the lack of thermal stability at high temperatures.

SUMMARY

A poly-GMA brush was readily anchored on the pore surface of a porous hollow-fiber membrane by radiation-induced graft polymerization. Introduction of functional groups and ligands into the epoxy group of the polymer brush provided various functions, such as multilayer binding of proteins and chelate formation with metal ions. This finding of protein multilayering by the charged polymer brush is expected to promote fundamental studies of the charged polymer brush and enable novel technological applications.

Radiation-induced graft polymerization is applicable to materials of various shapes, such as hollow-fiber membrane, film (39), and non-woven fabric (40). Moreover, other vinyl monomers, such as acrylonitrile and styrene as precursors for functionalization can be used for grafting. Functional materials with the polymer brush have been reported for the recovery of polyunsaturated fatty acid (41), collection of uranium from seawater (42), and removal of metal ions from ultrapure water (43).

REFERENCES

1. Halperin, A.; Tirrell, M.; Lodge, T.P. Tethered Chains in Polymer Microstructures. Adv. Polym. Sci. **1992**, *100*, 31–71.
2. Milner, S.T. Polymer Brushes. Science **1991**, *251*, 905–914.
3. de Gennes, P.G. Conformations of Polymers Attached to an Interface. Macromolecules **1980**, *13*, 1069–1075.

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4. Halperin, A. Polymer Brushes that Resist Adsorption of Model Proteins: Design Parameters. Langmuir **1999**, *15*, 2525–2533.
5. Klein, J. Forces Between Mica Surfaces Bearing Layers of Adsorbed Polystyrene in Cyclohexane. Nature **1980**, *288*, 248–250.
6. Fytas, G.; Anastasiadis, S.H.; Seghrouchni, R.; Vlassopoulos, D.; Li, J.; Factor, B.J.; Theobald, W.; Toprakcioglu, C. Probing Collective Motions of Terminally Anchored Polymers. Science **1996**, *274*, 2041–2044.
7. Karim, A.; Douglas, J.F.; Horkay, F.; Fetters, L.J.; Satija, S.K. Comparative Swelling of Gels and Polymer Brush Layers. Physica B **1996**, *221*, 331–336.
8. Roters, A.; Schimmel, M.; Ruhe, J.; Johannsmann, D. Collapse of a Polymer Brush in a Poor Solvent Probed by Noise Analysis of a Scanning Force Microscope Cantilever. Langmuir **1998**, *14*, 3999–4004.
9. Habicht, J.; Schmidt, M.; Ruhe, J.; Johannsmann, D. Swelling of Thick Polymer Brushes Investigated with Ellipsometry. Langmuir **1999**, *15*, 2460–2465.
10. Zhulina, E.B.; Israels, R.; Fleer, G.J. Theory of Planar Polyelectrolyte Brush Immersed in Solution of Asymmetric Salt. Colloids Surf. A **1994**, *86*, 11–24.
11. Israels, R.; Leermarkers, F.A.M.; Fleer, G.J.; Zhulina, E.B. Charged Polymer Brushes: Structure and Scaling Relations. Macromolecules **1994**, *27*, 3249–3261.
12. Saito, K.; Tsuneda, S.; Kim, M.; Kubota, N.; Sugita, K.; Sugo, T. Radiation-Induced Graft Polymerization is the Key to Develop High-Performance Functional Materials for Protein Purification. Radiat. Phys. Chem. **1999**, *54*, 517–525.
13. Tsuneda, S.; Saito, K.; Furusaki, S.; Sugo, T. High-Throughput Processing of Proteins Using a Porous and Tentacle Anion-Exchange Membrane. J. Chromatogr. A **1995**, *689*, 211–218.
14. Yamagishi, H.; Saito, K.; Furusaki, S.; Sugo, T.; Ishigaki, I. Introduction of a High-Density Chelating Group into a Porous Membrane Without Lowering the Flux. Ind. Eng. Chem. Res. **1991**, *30*, 2234–2237.
15. Ito, Y. Signal-Responsive Permeation Through Porous Membranes Grafted with Polymer Brushes. Kobunshi Ronbunshu **1998**, *55*, 171–181.
16. Tsuneda, S.; Kagawa, H.; Saito, K.; Sugo, T. Hydrodynamic Evaluation of Three-Dimensional Adsorption of Protein to a Polymer Chain Grafted onto a Porous Substrate. J. Colloid Interf. Sci. **1995**, *176*, 95–100.
17. Kubota, N.; Kounosu, M.; Saito, K.; Sugita, K.; Watanabe, K.; Sugo, T. Control of Phenyl-Group Site Introduced on the Graft Chain for Hydrophobic Interaction Chromatography. React. Polym. **1996**, *29*, 115–122.

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18. Iwata, H.; Saito, K.; Furusaki, S.; Sugo, T.; Okamoto, J. Adsorption Characteristics of an Immobilized Metal Affinity Membrane. Biotechnol. Prog. **1991**, *7*, 412–418.
19. Kubota, N.; Konno, Y.; Miura, S.; Saito, K.; Sugita, K.; Watanabe, K.; Sugo, T. Comparison of Two Convection-Aided Protein Adsorption Methods Using Porous Membranes and Perfusion Beads. Biotechnol. Prog. **1996**, *12*, 869–872.
20. Uezu, K.; Saito, K.; Furusaki, S.; Sugo, T.; Ishigaki, I. Radicals Contributing to Preirradiation Graft Polymerization onto Porous Polyethylene. Radiat. Phys. Chem. **1992**, *40*, 31–36.
21. Tsuneda, S.; Saito, K.; Furusaki, S.; Sugo, T.; Ishigaki, I. Water/Acetone Permeability of Porous Hollow-Fiber Membrane Containing Diethylamino Groups on the Grafted Polymer Branches. J. Membr. Sci. **1992**, *71*, 1–12.
22. Kim, M.; Kojima, J.; Saito, K.; Furusaki, S.; Sugo, T. Reduction of Nonselective Adsorption of Proteins by Hydrophilization of Microfiltration Membranes by Radiation-Induced Grafting. Biotechnol. Prog. **1994**, *10*, 114–120.
23. Matoba, S.; Tsuneda, S.; Saito, K.; Sugo, T. Highly Efficient Enzyme Recovery Using Porous Membrane with Immobilized Tentacle Polymer Chains. Biol. Technol. **1995**, *13*, 795–797.
24. Kawai, T.; Saito, K.; Sugita, K.; Sugo, T. Extension and Shrinkage of Polymer Brush Grafted onto Porous Membrane Induced by Protein Binding. Macromolecules **2000**, *33*, 1306–1309.
25. Tsuneda, S.; Shinano, H.; Saito, K.; Furusaki, S.; Sugo, T. Binding of Lysozyme onto a Cation-Exchange Microporous Membrane Containing Tentacle-Type Grafted Polymer Branches. Biotechnol. Prog. **1994**, *10*, 76–81.
26. Sasagawa, N.; Saito, K.; Sugita, K.; Kunori, S.; Sugo, T. Ionic Crosslinking of SO_3H-Group-Containing Graft Chains Helps to Capture Lysozyme in a Permeation Mode. J. Chromatogr. A **1999**, *848*, 161–168.
27. Tsuneda, S.; Saito, K.; Sugo, T.; Makuuchi, K. Protein Adsorption Characteristics of Porous and Tentacle Anion-Exchange Membrane Prepared by Radiation-Induced Graft Polymerization. Radiat. Phys. Chem. **1995**, *46*, 239–245.
28. Brandt, S.; Goffe, R.A.; Kessler, S.B.; O'Connor, J.L.; Zale, S.E. Membrane-Based Affinity Technology for Commercial Scale Purifications. Biol. Technol. **1988**, *6*, 779–782.
29. Muller, W. New Ion Exchangers for the Chromatography of Biopolymers. J. Chromatogr. **1990**, *510*, 133–140.
30. Sasagawa, N.; Saito, K.; Sugita, K.; Ogasawara, T.; Sugo, T. Adsorption Characteristics of Binary Proteins onto Anion-Exchange Porous Hollow-Fiber Membrane. J. Ion Exch. **1998**, *9*, 74–80.

MARCEL DEKKER, INC.
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31. Allenmark, S.G.; Bomgrem, B. Direct Liquid Chromatographic Separation of Enantiomers on Immobilized Protein Stationary Phases: III. Optical Resolution of a Series of *N*-Aroyl D,L-Amino Acid by High-Performance Liquid Chromatography on Bovine Serum Albumin Covalently Bound to Silica. J. Chromatogr. **1983**, *264*, 63–68.
32. Nakamura, M.; Kiyohara, S.; Saito, K.; Sugita, K.; Sugo, T. Chiral Separation of DL-Tryptophan Using Porous Membranes Containing Multilayered Bovine Serum Albumin Crosslinked with Glutaraldehyde. J. Chromatogr. A **1998**, *822*, 53–58.
33. Nakamura, M.; Kiyohara, S.; Saito, K.; Sugita, K.; Sugo, T. High Resolution of DL-Tryptophan at High Flow Rates Using a Bovine Serum Albumin-Multilayered Porous Hollow-Fiber Membrane. Anal. Chem. **1999**, *71*, 1323–1325.
34. Kawai, T.; Nakamura, M.; Sugita, K.; Saito, K.; Sugo, T. High Conversion in Asymmetric Hydrolysis During Permeation Through Enzyme-Multilayered Porous Hollow-Fiber Membranes. Biotechnol. Prog. **2001**, *17*, 872–875.
35. Yokote, Y.; Fujita, M.; Shimura, G.; Noguchi, S.; Kimura, K.; Samejima, H. Immobilized Aminoacylase on Porous Glass Beads. J. Solid-Phase Biochem. **1976**, *1*, 1–13.
36. Nakamura, M.; Saito, K.; Sugita, K.; Sugo, T. Application of Crosslinked-Aminoacylase-Multilayered Membranes to Bioreactor. Maku (Membrane) **1998**, *23*, 316–321.
37. Koguma, I.; Sugita, K.; Saito, K.; Sugo, T. Multilayer Binding of Proteins to Polymer Chains Grafted onto Porous Hollow-Fiber Membranes Containing Different Anion-Exchange Groups. Biotechnol. Prog. **2000**, *16*, 456–461.
38. Kubota, N.; Konno, Y.; Saito, K.; Sugita, K.; Watanabe, K.; Sugita, T. Module Performance of Anion-Exchange Porous Hollow-Fiber Membranes for High-Speed Protein Recovery. J. Chromatogr. A **1997**, *782*, 159–165.
39. Tsuneda, S.; Saito, K.; Mitsuhara, H.; Sugo, T. Novel Ion-Exchange Membranes for Electrodialysis Prepared by Radiation-Induced Graft Polymerization. J. Electrochem. Soc. **1995**, *142*, 3659–3663.
40. Kim, M.; Sasaki, M.; Saito, K.; Sugita, K.; Sugo, T. Protein Adsorption Characteristics of a Sulfonic-Acid-Containing Nonwoven Fabric. Biotechnol. Prog. **1998**, *14*, 661–663.
41. Shibasaki, A.; Irimoto, Y.; Kim, M.; Saito, K.; Sugita, K.; Baba, T.; Honjyo, I.; Moriyama, S.; Sugo, T. Selective Binding of Docosahexaenoic Acid Ethyl Ester to a Silver-Ion-Loaded Porous Hollow-Fiber Membrane. J. Am. Oil Chem. Soc. **1999**, *76*, 771–775.

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42. Katakai, A.; Seko, N.; Kawakami, T.; Saito, K.; Sugo, T. Adsorption of Uranium in Sea Water Using Amidoxime Adsorbents Prepared by Radiation-Induced Cografting. J. At. Energy Soc. Jpn **1998**, *40*, 878–880.
43. Hori, T.; Hashino, M.; Omori, A.; Matsuda, T.; Takasa, K.; Watanabe, K. Synthesis of Novel Microfilters with Ion-Exchange Capacity and Its Application to Ultrapure Water Production Systems. J. Membr. Sci. **1997**, *132*, 203–211.

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