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Review

Protein binding to polymer brush, based on ion-exchange, hydrophobic, and affinity interactions

Tomomi Kawai[a], Kyoichi Saito[a,*], William Lee[b]

[a]*Department of Materials Technology, Chiba University 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*
[b]*eMembrane, 129 Franklin Street, Suite 207, Cambridge, MA 02139, USA*

Abstract

The major limitations associated with conventional packed bed chromatography for protein separation and purification can be overcome by using adsorptive microporous membranes as chromatographic media. Microporous membranes have advantages as support matrices in comparison to conventional bead supports because they are not compressible and they eliminate diffusion limitations. As a result, higher throughput and shorter processing times are possible using these membrane systems. In this paper, we review the current state of development in the area of attaching functionalized polymer brushes onto a microporous membrane to form a novel chromatographic medium for protein separation and purification. The functionalized polymer brushes were appended onto the pore surface of a microporous hollow-fiber membrane uniformly across the membrane thickness by radiation-induced graft polymerization and subsequent chemical modifications. We review various applications of this adsorptive membrane chromatography by focusing on polymer brushes bearing ion-exchange, hydrophobic and affinity groups. Proteins were captured in multilayers by the ion-exchange group-containing polymer brushes due to the formation of a three-dimensional space for protein binding via the electrostatic repulsion of the polymer brushes. In contrast, proteins were captured in a monolayer at most by the polymer brushes containing hydrophobic or affinity ligands. By permeating a protein solution through the pores rimmed by the polymer brushes, an ideal capturing rate of the proteins with a negligible diffusional mass-transfer resistance was achieved by the functionalized polymer brushes, based on ion-exchange, hydrophobic, and affinity interactions.

Keywords: Reviews; Hollow-fiber membranes; Proteins

Contents

*Corresponding author. Tel.: +81-432-903-439; fax: +81-432-903-439.
E-mail address: marukyo@xtal.tf.chiba-u.ac.jp (K. Saito).

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1. Introduction

Proteins are natural polymers consisting of amino acid residues in a specified sequence. Native proteins that are dissolved in human serum, such as albumin, globulin, and coagulation factors, have been fractionated using conventional gel bead chromatography based on various physicochemical interactions. Some proteins are serviceable for medicines. For example, lysozyme in hen egg white has been commercialized as a component of cold medicines, and recombinant proteins are currently being used for the treatment of various diseases.

Rapid development in biotechnology and the pharmaceutical potential of proteins are fueling the demand for reliable and efficient protein separation and purification methods. Separation and purification of fragile proteins from a biological host environment require attention to their unique characteristics. For example, time-consuming separation and purification processes lead to unnecessary degradation of many recombinant products, whereas mild processing conditions help to maintain the native conformation and hence the biological activity of proteins. Additional considerations arise as trace amounts of target proteins have to be prepared in sufficient amounts for evaluation as drug candidates. Selected purification methods must consistently remove potentially hazardous variants and high resolution is required to meet stringent purification standards set for recombinant protein products.

Large-scale separation and purification processes must be efficient because the cost of protein recovery can dominate total product manufacturing costs [1]. In a typical protein production process from fermentation to final production, the steps of recovery, separation and purification account for 50 to 90% of the total cost of the bioprocess. This process requires multiple steps of filtration and chromatography and some proteins undergo as many as 10 different procedures to achieve its final purified form. Generally, there are microfiltration/ultrafiltration steps using membrane filters, followed by protein binding steps using chromatography resins. Moreover, large-scale chromatography columns of standard packed gel beads require significant volumes of validated buffers and regeneration solutions. In addition to the expense of producing these solutions, there are significant costs associated with the storage and disposal of these solutions.

A chromatography column or bead-packed bed is by far the most convenient apparatus for separating and purifying proteins. Conventionally, various chromatographic beads have been commercialized by many manufacturers. However, conventional packed beds have several major limitations. The pressure drop across a packed bed is generally high. Another major limitation is the dependence on intra-particle diffusion transport of proteins to their binding sites within the pores of such beads. As a result, in order to speed up the protein separation and purification process, various chromatographic modes and techniques using beads selective or specific for proteins have been extensively developed [2].

Selectivity and specificity in protein binding are based on electrostatic or ion-exchange, hydrophobic, and affinity interactions between the adsorbent surface and the protein surface. Since the aforementioned cost of protein separation and purification accounts for a greater part of the total production cost, chromatography beads should continuously be improved for higher selectivity and specificity. However, as long as the chromatography medium is in the bead form, the major limitation of pressure drop remains.

In this review, the current state of development of adsorptive microporous membranes bearing functionalized polymer brushes is discussed. These membranes are prepared by the radiation-induced graft polymerization technique. The interaction modes investigated include ion-exchange, hydrophobic and affinity interactions. As the minimum requirements of the materials for protein purification are high rate, high capacity, high durability, and repeated use from

a material viewpoint, and low operating pressure and easy scale-up from the process viewpoint, the grafting of functionalized polymer brushes to a microporous hollow-fiber membrane is expected to simultaneously achieve the above requirements. The potential limitations of these adsorptive microporous membranes are also highlighted. This review also aims to enable the reader to use these concepts for purifying different proteins, particularly at a large scale, and for investigating new adsorptive microporous membranes as well as promoting a wider acceptance of this technology.

2. Radiation-induced graft polymerization

In this multistep process, common polymer materials, such as polyethylene and polypropylene in various shapes and sizes, are exposed to an electron beam that generates highly reactive radicals in the entire volume of the base material. These radicals are used as starting sites for the polymerization and extension of long polymer brushes from the bulk towards and off the surface of the materials. When an electron beam-treated material is reacted with a vinyl monomer, such as glycidyl methacrylate (GMA), GMA molecules will polymerize from the starting radical sites to form long poly-GMA brushes on the surface of the material.

The radiation-induced grafting technology can be used to control the density of the polymer brushes simply by varying the time that the base material is exposed to the electron beam. The length of the polymer brushes can also be easily controlled simply by varying the time that the treated base material is allowed to react with the vinyl monomer. Brush length can be controlled over a range of tens of nanometers to the micrometer scale.

Another important feature that can be controlled using this platform technology is the chemical composition of the polymer brushes. There is a large and varied selection of monomers, each with unique chemical properties that can be used to polymerize brushes. The choice of the monomer influences the chemical properties of the final material or membrane. In addition, many monomers can be chemically modified further during a functionalization step to impart on the brushes important properties such as the ability to bind specific molecules or ligands. For example, the reaction of a polyethylene membrane containing poly-GMA brushes with diethylamine (DEA) converts the epoxy groups into diethylamino groups ($-NH(C_2H_5)_2$), an anion-exchange group. The reaction with sodium sulfite (SS) converts the epoxy groups into sulfonic acid groups ($-SO_3H$), a cation-exchange group. This functionalization step is another important point of control in the platform technology and is easily altered by varying the reaction time.

For the preparation of the functional microporous hollow-fiber membranes, the degree of GMA grafting and the molar conversion of the epoxy group into a functional group were defined as follows:

$$\text{Degree of GMA grafting} = 100\,(\text{mass of polymer brushes})/(\text{mass of base membrane}) \quad (1)$$

$$\text{Molar conversion} = 100\,(\text{moles of functional group})/(\text{moles of epoxy group before functionalization}) \quad (2)$$

3. Hydrophilization of a hydrophobic microporous hollow-fiber membrane

Typical conventional beads, such as agarose and Sepharose, are based on hydrophilic polymers in order to reduce nonselective or irreversible adsorption of proteins. As we use a polyethylene-based membrane, the nonselective or irreversible adsorption of proteins onto the polymer surface must also be reduced. Hydrophilic polymer brushes bearing alcoholic hydroxyl groups were introduced onto the polyethylene surface by radiation-induced grafting of 2-hydroxyethyl methacrylate (HEMA), vinyl acetate (VAc), and GMA. The VAc-grafted and GMA-grafted membranes were hydrolyzed into a monohydroxyl group and a diol or two adjacent hydroxyl groups, respectively. With increasing amounts of the hydrophilic polymer brushes, the amount of γ-globulin adsorbed onto the membrane decreased and leveled off when the hydrophilic group density exceeded 7 mol/kg of the base membrane. In addition, the protein adsorbed onto the base membrane was not

completely eluted with 0.5 *M* sodium chloride, whereas the protein adsorbed onto the hydrophilized membrane was quantitatively eluted with 0.5 *M* sodium chloride. This demonstrates that the pore surface of the microporous hollow-fiber membrane was covered by the polymer brush containing a sufficient number of alcoholic hydroxyl groups to reduce the nonselective adsorption of the proteins [3].

Moreover, the hydrophilized polymer brushes bearing multiple glycol groups (from GMA) and alcohol groups (from VAc) provide an attractive base for linkage of ligands using chemistries developed with Sepharose gels. Since the polyethylene-based membrane is resistant to solvents, this functionalized membrane offers an unlimited variety of organic synthesis routes for further modification.

4. Permeation mode using microporous hollow-fiber membranes

Proteins dissolved in a buffer solution exhibit the characteristics of macromolecular ions depending on the pH of the solution. A protein behaves as positive or negative ions at pH lower or higher than its isoelectric point (p*I*), respectively. Therefore, charged or ion-exchange materials have been used for protein purification. An introduction scheme of the ion-exchange groups into the polymer brush is shown in Fig. 1a. Diethylamino and sulfonic acid groups were introduced into the poly-GMA brush grafted onto the microporous hollow-fiber membrane by immersion of the GMA-grafted microporous hollow-fiber membrane in diethylamine and sodium sulfite solution, respectively [4].

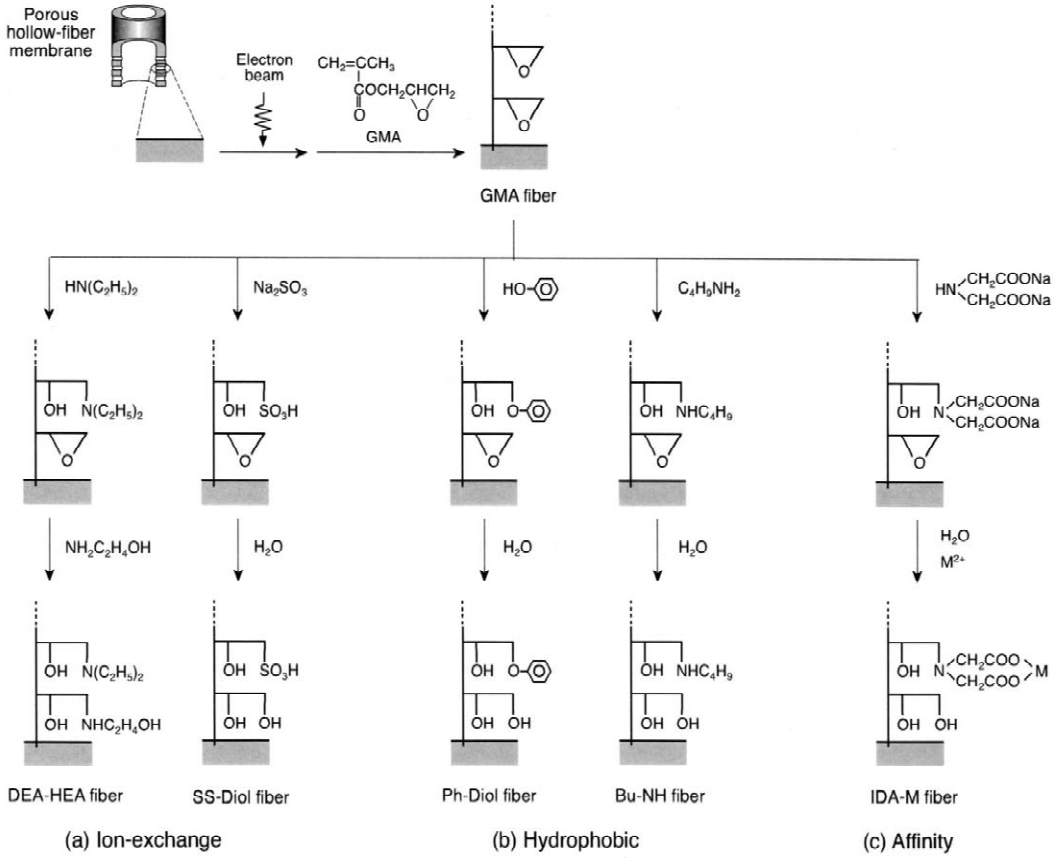


Fig. 1. Preparation scheme of functional polymer brush grafted onto the pore surface of a porous hollow-fiber membrane.

A permeation mode was adopted to evaluate the protein binding performance of the ion-exchange microporous hollow-fiber membranes [5,6]. The hollow fiber was positioned in a U-shaped configuration, as shown in Fig. 2. A protein solution with a constant concentration was fed to the inside surface of the ion-exchange microporous hollow-fiber membrane, and forced to permeate through the pores, driven by a transmembrane pressure. Either constant pressure or constant permeation rate was maintained with a peristaltic pump or a syringe infusion pump, respectively. The effluent penetrating the outside surface of the ion-exchange microporous hollow-fiber membrane was continuously collected to determine the protein concentration of the effluent.

A breakthrough curve, i.e., a change in protein concentration of the effluent as a function of effluent volume, was determined in the permeation mode. Initially, all proteins were captured by the ion-exchange polymer brush grafted onto the pore surface; the protein concentration was zero. With an increase in effluent volume, however, the protein concentration was found to gradually increase. Finally, the effluent concentration reached the feed concentration: adsorption equilibrium was attained. Then, the adsorption procedure was switched to washing and elution.

The equilibrium binding capacity (EBC) or the amount of protein adsorbed in equilibrium with the feed concentration was evaluated as follows:

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

where C_0 and C are the protein concentrations of the feed and the effluent, respectively. V, V_e and W are the effluent volume, the effluent volume when C reaches C_0, and the mass of the ion-exchange microporous hollow-fiber membrane. The degree of protein multilayering was defined as the ratio of equilibrium binding capacity to theoretical monolayer binding capacity as follows:

$$\text{Degree of protein multilayering} = \text{EBC}/(\text{theoretical monolayer binding capacity}) \tag{4}$$

After washing the pores with the buffer solution, the feed was switched to 0.5 or 1 M sodium chloride to elute the amount of protein adsorbed onto the ion-exchange polymer brush. Here, the elution percentage was defined as:

$$\mathrm{EP}\ (\%) = 100\ (\text{amount eluted})/[(\text{amount adsorbed}) - (\text{amount washed})] \tag{5}$$

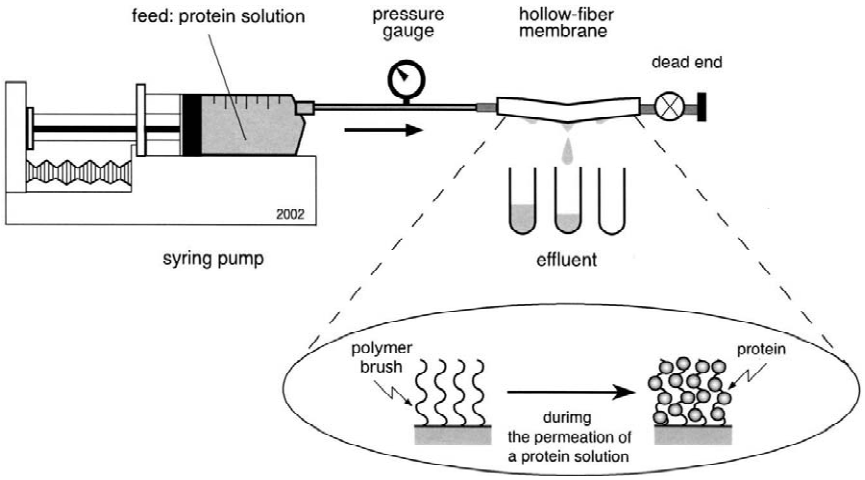


Fig. 2. Protein capturing during the permeation of a protein solution through the pores of a functional porous hollow-fiber membrane.

This method for evaluating protein binding performance is called ‘‘frontal analysis’’, and has been used in the quantitative evaluation of the bead-packed bed.

5. Anion-exchange interaction

The anion-exchange polymer brush containing the diethylamino (DEA) groups captures bovine serum albumin (*M*r 68 000; p*I* 4.9) dissolved in Tris–HCl buffer (pH 8.0). Some of the epoxy groups on the grafted poly-GMA brush with a degree of GMA grafting of 160% were converted into various anion-exchange groups such as amino ($-NH_2$), ethylamino ($-NHC_2H_5$), and diethylamino [$-N(C_2H_5)_2$] groups at various densities or molar conversions. The remaining epoxy groups were converted into 2-hydroxyethylamino groups ($-NHC_2H_5OH$). The resultant microporous hollow-fiber membrane was referred to as AM–HEA, EA–HEA, and DEA–HEA fibers, where AM, EA, DEA, and HEA designate amino, ethylamino, diethylamino, and 2-hydroxyethylamino groups, respectively. The equilibrium binding capacities of the anion-exchange microporous hollow-fiber membranes are shown in Fig. 3 as a function of molar conversion [7]. The equilibrium binding capacity of the AM–HEA fiber remained constant regardless of the molar conversion of the anion-exchange group, whereas, the equilibrium binding capacities of the EA–HEA and DEA–HEA fibers rapidly increased when the molar conversion of the anion-exchange group is higher than 60%. This demonstrates that a three-dimensional space for protein multilayering is induced by the increase in the density of the positively charged groups of the polymer brushes.

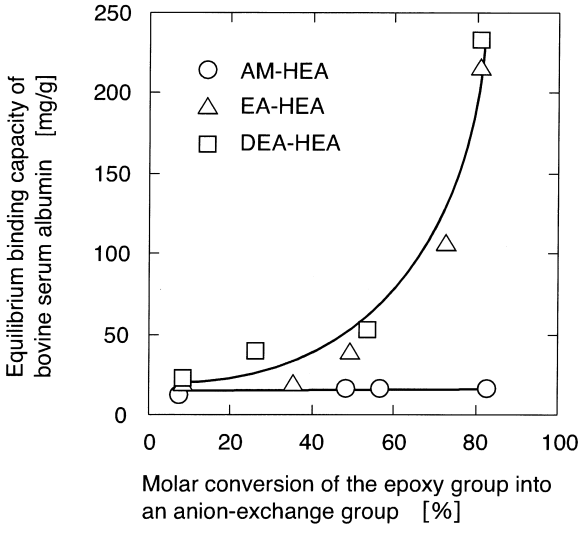


Fig. 3. Equilibrium binding capacity of bovine serum albumin vs. molar conversion of the epoxy group into an anion-exchange group.

Epoxy and 2-hydroxyethylamino groups as coexisting groups with the anion-exchange groups were compared in terms of equilibrium binding capacity and elution percentage. The equilibrium binding capacities were not markedly different between the DEA–EO and DEA–HEA fibers, where EO is the epoxy group coexisting with the anion-exchange group. On the other hand, the elution percentage of the DEA–EO fiber did not reach 100% below a molar conversion of 50%, whereas that of the DEA–HEA fiber was 100% regardless of the molar conversion; the HEA group reduced the amount of protein adsorbed nonselectively [7].

Convective flow of the protein solution through the pores across the anion-exchange microporous hollow-fiber membrane minimizes the diffusional path of the protein to the anion-exchange group on the polymer brush [8–12]. Breakthrough curves of bovine serum albumin determined in the permeation mode using the anion-exchange microporous hollow-fiber membrane are shown in Fig. 4 for various permeation rates [8]. The abscissa is the dimensionless effluent volume defined as the ratio of the effluent volume to the membrane volume excluding the lumen part. The breakthrough curves overlapped regardless of the permeation rate, i.e., the residence time of the protein solution. The residence time was defined as:

$$\text{Residence time (s)} = \epsilon\pi(r_o^2 - r_i^2)\,L/(\text{permeation rate}) \quad (6)$$

where r_o, r_i, L, and ϵ are the outer and inner radii, length, and porosity of the anion-exchange microporous hollow-fiber membrane, respectively. This is indicative of the negligible diffusional mass-transfer resistance of the protein to the anion-exchange group.

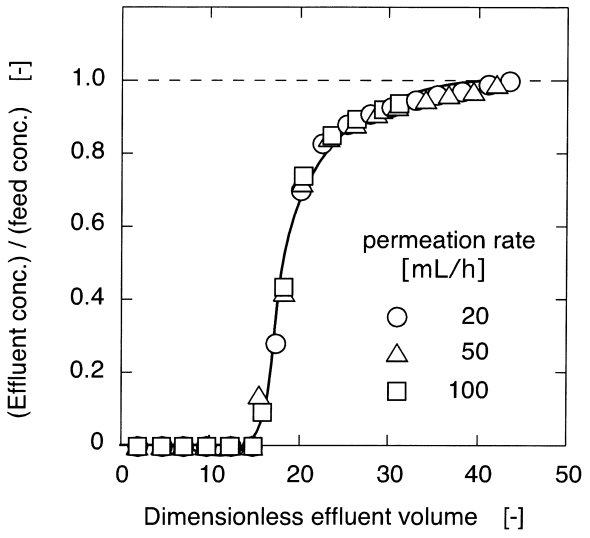


Fig. 4. Breakthrough curves of bovine serum albumin for the DEA–HEA fiber for various permeation rates of the protein solution.

The elution process as well as the adsorption process is crucial for concentrating the target protein. Elution is performed by permeating a high concentration of sodium chloride solution through the anion-exchange microporous hollow-fiber membrane. The advantage of protein transport aided by the convective flow in adsorption holds true for elution; proteins released by the polymer brush can be transported by the convective flow of the eluent through the pores. Therefore, tailing in the elution curve is minimized to significantly increase the concentration factor of the protein. However, with increasing degree of protein multilayering, the tailing in the elution curve appears because the diffusion of the protein released by the polymer brush at a high concentration into the eluent partly governs the overall elution rate of the protein [13].

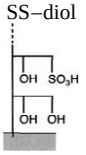
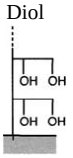
6. Cation-exchange interaction

Cation-exchange materials are necessary to recover proteins with higher p*I* values such as lysozyme (p*I* 10) and avidin (p*I* 11). Similar to the preparation of the anion-exchange microporous hollow-fiber membranes, the GMA-grafted microporous hollow-fiber membrane was used to introduce a cation-exchange group into the poly-GMA brush [14,15]; the epoxy group was readily converted into the sulfonic acid group, a strongly acidic cation-exchange group, by reacting with sodium sulfite dissolved in isopropyl alcohol/water mixture [15,16]. The molar conversion ranged from 10 to 90% by changing the reaction time at 353 K. The remaining epoxy groups were converted into the diol group to reduce nonselective adsorption of the proteins. The resultant microporous hollow-fiber membrane was referred to as an SS–diol membrane.

The strongly acidic cation-exchange group of the polymer brush enhances electrostatic repulsion more than the weakly basic anion-exchange groups of the polymer brush. Even at a molar conversion of 10%, the flux of the SS–diol membrane was almost zero. Although the SS–diol membrane has a potential to bind the proteins based on electrostatic interaction, the negligible flux of the protein solution leads to non recovery of the protein. In order to improve the flux of the SS–diol membrane, prior to protein adsorption, a solution containing bivalent ions, such as Mg^{2+} and Ca^{2+}, was forced to permeate through the pores to crosslink the cation-exchange polymer brush: the extension of the sulfonic acid group-containing polymer brush was restricted via ionic crosslinking. Table 1 shows the effect of alkaline and alkaline-earth metal ions on flux increase [16]. The crosslinking by the bivalent ions markedly increased the liquid permeability of the SS–diol membrane. Subsequently, when a lysozyme solution (pH 8.0) was permeated across the SS–diol membrane, magnesium ion was replaced with lysozyme because the latter has a higher affinity for the sulfonic acid group than the former. In other words, instead of the magnesium ions, the lysozyme crosslinked the polymer brush via multipoint binding to increase the flux (Fig. 5). From the breakthrough curve of lysozyme for the SS–diol membrane, the equilibrium binding capacity was evaluated to be 300 g of lysozyme per kilogram of the SS–diol membrane, which was equivalent to the degree of protein multilayering of 38.

Protein multilayering or binding of proteins in multilayers, by the charged polymer brush is not an exceptional phenomenon. Muller [17] suggested the grafting of ion-exchange group-containing vinyl monomers onto the external surface of the polymeric

Table 1
Liquid flux for sulfonic acid-containing porous hollow-fiber membrane (0.06 MPa, 298 K)

	SS–diol	Diol	PE
			
Liquid	Liquid flux [m/h]		
Water	0.10	1.9	3.1
0.005 *M* NaCl	0.19	2.0	3.1
0.005 *M* KCl	0.16	2.0	3.1
0.005 *M* $CaCl_2$	1.50	2.0	3.1
0.005 *M* $MgCl_2$	1.20	2.0	3.1

beads to increase the protein binding capacity, and named the protein multilayering ''tentacle binding''. Both positively and negatively charged polymer brushes can hold the proteins in multilayers, as shown in Table 2. Regardless of the molecular mass and the isoelectric point, the degree of protein multilayering was observed [8,18]. By multiplying the size of the protein with the degree of protein multilayering, the effective length of the polymer brush grafted onto the pore surface of the ion-exchange microporous hollow-fiber membrane was estimated to be of the order of 1 μm.

The epoxy group-containing polymer brush grafted onto the microporous hollow-fiber membrane prepared by radiation-induced graft polymerization worked well for convection-aided protein recovery. First, a high-rate operation was achievable by convection instead of by diffusion, driven by transmembrane pressure; the microporous form of the base membrane played a decisive role in improving the mass-transfer characteristics. Second, a high-capacity operation was achievable due to the introduction of charged or ion-exchange groups into the polymer brush, which may have an extended length

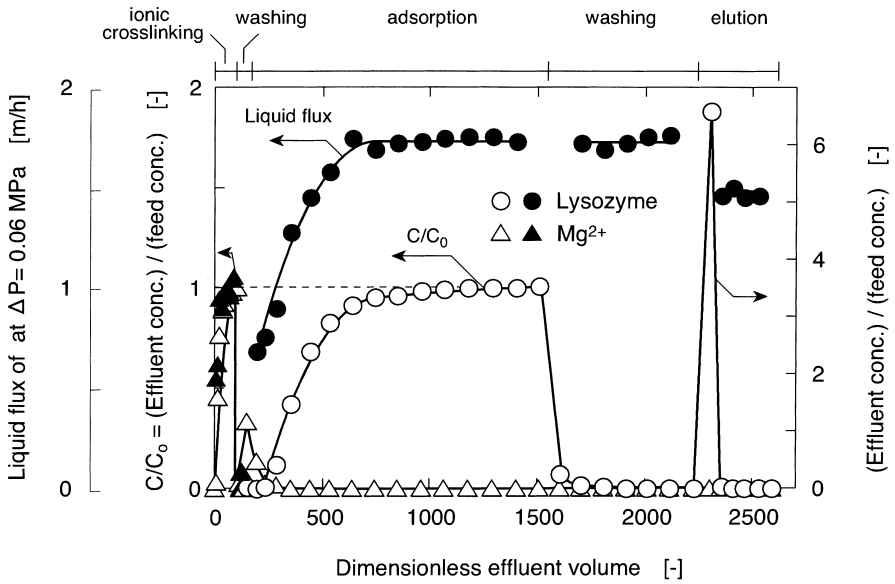


Fig. 5. Changes of flux and protein concentration of the effluent penetrating the SS–diol fiber.

Table 2
Protein multilayer binding onto the charged polymer brushes grafted onto a porous hollow-fiber membrane

Functional moiety	Protein	Degree of multilayer binding	Ref.
EA–HEA	β-lactoglobulin	12.0	[7]
	BSA	3.6	
	Urease	32.0	
DEA–HEA	β-lactoglobulin	12.0	[7]
	BSA	4.0	
	Urease	44.0	
	Bovine γ-globulin	4.1	[8]
	Aminoacylase	7.0	[35]
	Ascorbic acid oxidase	12.0	[37]
	CITase	3.0	[38]
SS–diol	Lysozyme	38.0	[16]

of 1 μm; the electrostatic repulsion among the charged polymer brush provided a three-dimensional space for protein binding in which the proteins can travel into the ''jungle'' of the extending polymer brush. In addition, a high durability for repeated adsorption and elution of the protein was attainable because some of the epoxy groups were converted into anion-exchange groups to enhance selective adsorption of the target protein, and the remaining epoxy groups were converted into hydrophilic groups to reduce nonselective adsorption of undesired proteins.

7. Hydrophobic interaction

A protein surface has various patches such as charged or noncharged patches, and hydrophilic or hydrophobic patches. Hydrophobic interaction between proteins and the adsorptive materials is strengthened by the addition of inorganic salts at high concentrations, e.g., 2 *M* ammonium sulfate, to a protein solution. Phenyl and butyl groups were introduced into the polymer brush by reacting some of the epoxy groups on the polymer brush with phenol and butanol, respectively [19–22]. The remaining epoxy groups were converted into diol groups to reduce nonselective adsorption of the proteins (Fig. 1b). The resultant microporous hollow-fiber membrane was referred to as Ph and Bu membranes. Bovine serum albumin dissolved in 2 *M* ammonium sulfate as a model protein solution was permeated through the pores of the Ph membrane at various permeation rates. The breakthrough curves of bovine serum albumin overlapped regardless of the permeation rate or the residence time of the protein solution [20,22]. This is because the protein is transported to the hydrophobic ligands by convective flow or permeation flow with negligible diffusional mass-transfer resistance; therefore, the higher permeation rate of the protein solution leads to the higher binding rate of the protein to the polymer brush, which is an ideal separation principle.

Neither the phenyl nor the butyl group allowed the polymer brush to extend from the pore surface of the hydrophobic microporous hollow-fiber membrane toward the pore interior. The equilibrium binding capacity of bovine serum albumin remained constant regardless of molar conversion or ligand density. As expected, the equilibrium binding capacity corresponded to the amount of bovine serum albumin adsorbed in a monolayer onto the pore surface immobilizing the hydrophobic polymer brush [20].

Protein was partly eluted from the hydrophobic polymer brush by decreasing the ionic strength, e.g., the concentration of ammonium sulfate; approximately 80% of adsorbed bovine serum albumin was eluted in each elution step. This is different from the elution of proteins adsorbed via the electrostatic interaction which enabled complete elution with 0.5 *M* sodium chloride. The immersion of the hydrophobic polymer brush in 2 *M* sodium hy-

droxide at ambient temperature for 2 h was effective in regaining the binding capacity of the phenyl group-containing polymer brush grafted onto the pore surface of the microporous hollow-fiber membrane [19].

8. Affinity interaction

Affinity interaction or biologically specific interaction, such as a pair of antigen and antibody or enzyme and substrate, has been utilized for protein purification. In principle, the affinity interaction is most powerful when a target molecule is needed to be isolated from complex liquids by minimum steps. The affinity interaction is ascribed to the integrated recognition of a molecule by the corresponding molecule based on size, electrostatic and hydrophobic interactions, metal-chelate and hydrogen bondings among others. Affinity ligands are classified into biospecific and pseudobiospecific ligands. The former ligands include monoclonal or polyclonal antibodies. The latter ligands include immobilized metals, hydrophobic amino acids, and dyes.

Some metals or metal ions exhibit a specific interaction via histidine residue or disulfide bonding. For example, copper, zinc, or nickel chelated by an iminodiacetate group can selectively bind to the histidine residue of the protein surface. The iminodiacetate group was introduced into the polymer brush via graft polymerization of glycidyl methacrylate and subsequent ring-opening reaction with disodium iminodiacetate. The iminodiacetate group-containing microporous hollow-fiber membrane was immersed in cupric chloride solution to bind copper ion (Fig. 1c). The resultant microporous hollow-fiber membrane exhibited pseudoaffinity for bovine serum albumin as a model protein [23]. The iminodiacetate group of the polymer brush grafted onto the pore surface and the histidine residue of the protein dissolved in the buffer compete for the copper ion; therefore, as the protein binding progressed, leakage of the copper ion from the iminodiacetate group was observed. As described in the ion-exchange and hydrophobic polymer brush, the permeation of a protein solution through the pores of the microporous hollow-fiber membrane helps to minimize the diffusional path of the protein to the pseudoaffinity ligand of the polymer brush.

Silver ions bound by the sulfonic acid group of the polymer brush show an affinity for polyunsaturated fatty acids (PUFAs) such as docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) because carbon–carbon double bonds of the PUFA molecule coordinate with the silver ions. DHA ethyl ester in bonito oil was purified in the permeation mode using the silver ion-immobilized microporous hollow-fiber membrane [24,25]. DHA ethyl ester was adsorbed onto the microporous hollow-fiber membrane in methanol, and eluted from the microporous hollow-fiber membrane in acetonitrile.

A hydrophobic amino acid as the pseudoaffinity ligand, such as tryptophan or phenylalanine, was immobilized to various polymeric materials to specifically remove disease-causing proteins from blood. Conventionally, the phenylalanine ligand-immobilized bead-packed bed has been used for such therapy. In order to improve the adsorption rate of the protein, phenylalanine or tryptophan was coupled to the epoxy group of the polymer brush grafted onto the microporous hollow-fiber membrane [26,27]. Bovine γ-globulin as a model protein was adsorbed onto the pseudoaffinity microporous hollow-fiber membrane. The equilibrium binding capacity of bovine γ-globulin was found to be between the two extremes of oriented bindings; end-on and side-in bindings onto the interface between the polymer brush and the liquid [27].

9. Conclusion

Protein recovery using functionalized microporous hollow-fiber membranes is much improved compared to that using conventional bead-packed beds in that a target protein is transported to various functional groups such as ion-exchange groups, and hydrophobic and affinity ligands by convective flow through the pores driven by the transmembrane pressure. In the case of permeating a protein solution through the pores rimmed by the functional polymer brush, the overall adsorption rate of the protein was accelerated with increasing permeation rate of the protein solution. When the intrinsic reaction between

a protein and a ligand, e.g., macromolecular antigen and antibody, is not instantaneous, the merit of using the microporous hollow-fiber membranes is reduced. However, the results described here proved otherwise.

The multilayer binding of proteins in the polymer brush was observed exclusively for the charged or ion-exchange polymer brush because the polymer brush extended itself from the pore surface toward the pore interior due to electrostatic repulsion. In contrast, monolayer binding capacity was observed in the hydrophobic and affinity interaction modes.

The adsorption and elution process of proteins were readily repeated for the ion-exchange groups of the polymer brush. In contrast, the protein binding capacity of hydrophobic- and affinity ligand-immobilized microporous hollow-fiber membranes deteriorated after repeated use. Regeneration of the hydrophobic ligand-containing microporous hollow-fiber membranes with an alkaline solution was effective in recovering the protein binding performance.

Low operational pressure and linear scale-up of the functionalized microporous hollow-fiber membranes were additional advantages over the functional bead-packed beds [28,29]. The adsorptive microporous hollow-fiber membrane is not a competitor of the bead-packed bed for protein separation and purification. Rather, it is an advanced and revolutionized form of the conventional bead-packed bed that leads to simple module fabrication by bundling the adsorptive microporous hollow-fiber membranes [30].

Multilayer binding of bovine serum albumin as a chiral selector was applicable to high-performance chiral separation [31–34]. Enzyme multilayer binding improved the overall activity of the enzymes such as aminoacylase [35,36], ascorbic acid oxidase [37], and cyclo-iso-malto-oligo-glucano-transferase [38,39] in the permeation mode. Convection-aided transport of target ions and molecules through the pores of the functionalized microporous hollow-fiber membranes can be applied to improve the removal rate of undesired metal ions such as cobalt ions in the cooling line of an atomic power plant [40–42] and improve the recovery rate of precious ions such as germanium species [43–45].

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