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High-throughput processing of proteins using a porous and tentacle anion-exchange membrane

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Abstract

The immobilization of polymer chains containing a diethylamino (DEA) group on the pore surface of a porous hollow-fibre membrane is reported. This novel membrane can collect proteins at a high rate and high capacity because of convective transport and multi-layering of proteins. Overlapping of the breakthrough curves for different residence times of bovine serum albumin (BSA) solution demonstrates that the diffusional resistance of BSA to the DEA group anchored to the polymer chain was negligible. Membranes with a higher density of DEA groups exhibited a higher binding capacity for BSA. For example, a membrane with a DEA group density of 2.9 mol/kg had a BSA binding capacity of 490 g/kg, which was equivalent to eleven times the adsorption capacity of a monolayer. This vertical layering is due to holding of the BSA molecules in a tentacle-like manner by the graft chains extending from the pore surface towards the pore interior.

1. Introduction

Conventional ion-exchange beads used for protein collection and purifications allow proteins to diffuse into the bead interior, immobilizing the ion-exchange groups; in this case, a longer time is required for proteins of higher molecular mass to reach the ion-exchange groups. This diffusion-limited binding process also results in a lower degree of enrichment in a subsequent elution process.

Brandt et al. [1] prepared a porous affinity membrane for the purification of proteins and demonstrated that convective transport of the protein minimized the diffusional path to the ligand. Afeyan et al. [2] prepared a functionalized bead having pores through which a protein can be transported by convection and showed that diffusional resistance in the bead-packed bed could be neglected. These two methods of chromatography aided by convection were designated membrane chromatography and perfusion chromatography, respectively.

We have anchored affinity ligands and ion-exchange groups to polymer chains grafted on the pore surface of porous membranes [3–5]. Negligible diffusional resistance of the protein to the ligand and ion-exchange group could be attained by permeating the protein solution through the porous membrane; a higher permeation rate led to a higher collection rate of the protein.

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Conventional ion-exchange beads exhibit a monolayer adsorption capacity for proteins, and therefore porous ion-exchange beads with a higher specific surface area have been used for preparative chromatography. On the other hand, Muller [6] suggested tentacle-type ion-exchange beads, which were prepared by chemical grafting of vinyl monomers originally containing ion-exchange groups. The ion-exchange group-containing polymer chains can thus hold the proteins three-dimensionally like a tentacle. Monomers and supporting materials for the tentacle polymer chains have been studied for various kinds of proteins [7]. In order to clarify the tentacle phenomenon capable of collecting proteins in multi-layers, the dependence of the degree of tentacle binding on the nature of the grafted polymer branches, such as length, number density and charge density of the polymer chain, should be elucidated.

In this study, we developed a novel "porous and tentacle" membrane that achieves protein processing at a high rate and high capacity. The porous membrane immobilizing the ion-exchange graft chain capable of tentacle binding can collect proteins at a high rate and high capacity because of convective transport and multi-layering of the proteins. Fig. 1 illustrates the principle of processing of proteins using the porous and tentacle membrane. A diethylamino (DEA) group [$-N(CH_2CH_3)_2$] as a weakly basic anion-exchange group was appended to a porous hollow-fibre membrane by radiation-induced graft polymerization and subsequent chemical modification. The DEA-containing anion exchanger has a wide applicability for protein collection [8–11]. Advantages brought about by the porous and tentacle membrane in protein collection were evaluated by permeating a protein solution through the pores of the resultant hollow fibre.

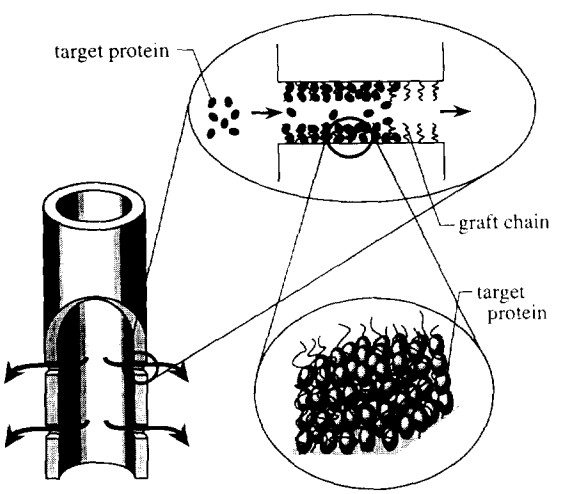

Fig. 1. Processing of proteins using the porous and tentacle ion-exchange membrane. Proteins are adsorbed on the ion-exchange group-containing polymer chain immobilized on the pore surface of the membrane. (a) Convection of the liquid across the hollow fibre through its pores minimizes the diffusion path of the proteins to the ion-exchange groups on the graft chain. (b) The graft chains extending towards the pore interior from the pore surface lead to vertical layering of proteins.

2. Experimental

2.1. Materials

A commercially available microfiltration hollow-fibre membrane (Asahi Chemical Industry, Tokyo, Japan) was used as a trunk polymer for grafting. This hollow fibre, made of polyethylene, has inner and outer diameters of 1.95 and 3.01 mm, respectively, with a nominal pore diameter of 0.34 μm and a porosity of 71%. Technical-grade glycidyl methacrylate [GMA, $CH_2=C(CH_3)COOCH_2CHOCH_2$] was purchased from Tokyo Kasei Industry (Tokyo, Japan) and used without further purification. Diethylamine [$NH(CH_2CH_3)_2$] and ethanolamine ($NH_2CH_2CH_2OH$) were obtained from Wako (Osaka, Japan). Lactoglobulin, bovine serum albumin and bovine γ-globulin were purchased from Sigma (St. Louis, MO, USA).

2.2. Preparation of diethylamino-containing membrane

Fig. 2 shows the preparation scheme for the DEA-containing hollow-fibre membrane. A 10-

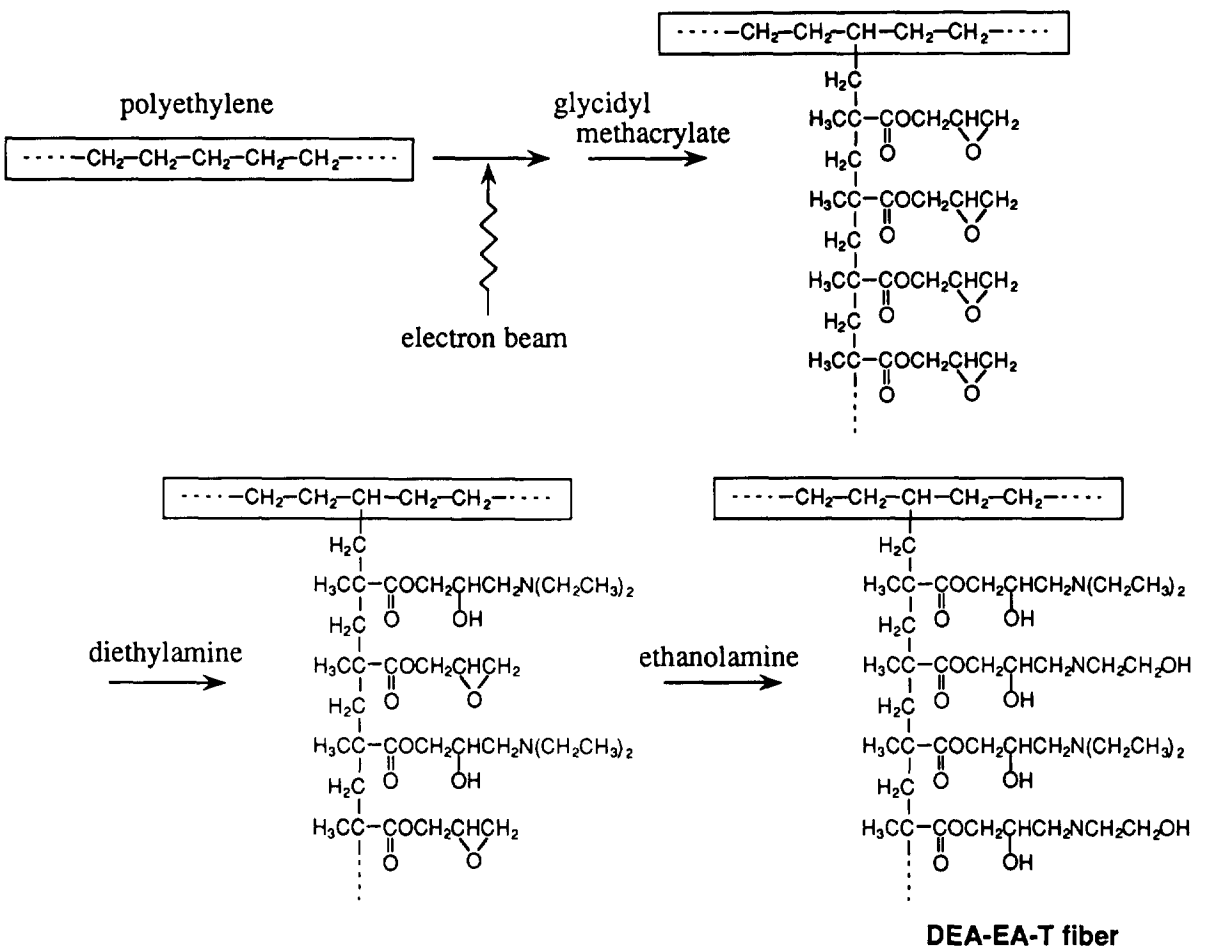

Fig. 2. Scheme of preparation of diethylamino-containing hollow-fibre membrane.

cm long hollow fibre was irradiated with an electron beam from an accelerator at ambient temperature under a nitrogen atmosphere. The total dose was 200 kGy. The irradiated hollow fibre was immersed at 313 K in 10% (v/v) solution of GMA in methanol which had previously been deaerated. The amount of GMA grafted on to the hollow fibre was expressed by the degree of grafting,

$$dg = 100[(M_1 - M_0)/M_0] \quad (1)$$

where M_0 and M_1 are the masses of the original and GMA-grafted hollow fibres, respectively. Here, dg was set at 200%.

The epoxy groups produced were converted into diethylamino (DEA) groups by immersing the GMA-grafted hollow fibre in 50% (v/v) aqueous diethylamine solution at 303 K. After the prescribed time, the hollow fibre was removed, washed repeatedly with deionized water and dried under reduced pressure. The remaining epoxy groups were subsequently converted into ethanolamino (EA) groups by soaking the hollow fibre in ethanolamine at 303 K for 6 h. The density of DEA groups was defined from the mass change as follows:

$$\text{density of DEA groups } (X) = [((M_2 - M_1)/73)/M_3] \quad (2)$$

where M_2 and M_3 are the masses of the DEA-

containing hollow fibre and DEA- and EA-containing hollow fibre, respectively. The constant 73 is the molecular mass of diethylamine.

2.3. Properties of membrane

The membrane volume V_m in the wet state was calculated from the equation

$$V_m = \pi L(d_o^2 - d_i^2)/4 \quad (3)$$

where d_i, d_o and L are the inner and outer diameters and the length of the hollow fibre, respectively. Prior to volume measurement, the hollow fibre was immersed in methanol for 10 min and subsequently replaced with water. The inner and outer diameters of the hollow fibre were measured under a microscope and the length with a scale.

After the hollow fibre had been dried under reduced pressure, the specific surface area was determined by the nitrogen adsorption method using Quantasorb (Yuasa Ionics, Tokyo, Japan). The pore structure was observed by scanning electron microscopy.

2.4. Adsorption and elution of proteins during permeation

The protein was dissolved in 0.02 *M* Tris–HCl buffer (pH 8.0). The feed concentration was 5 mg/ml. A hollow fibre was set in an I-configuration. The solution was forced to permeate from the inside to the outside of the hollow fibre at a constant flow-rate using an infusion syringe pump (ATOM-235, ATOM, Tokyo, Japan). The flow-rate ranged from 20 to 100 ml/h. The effluent penetrating the outside surface of the hollow fibre was collected using a fraction collector (Model 2110; Bio-Rad Labs., Richmond, CA, USA). The protein concentration of each fraction was determined by measuring the UV absorbance at 280 nm.

After adsorption equilibrium had been attained in the permeation mode, 10 ml of the protein-free buffer solution and 0.02 *M* Tris–HCl buffer solution containing 0.5 *M* NaCl were forced to permeate radially outwards to wash and elute the adsorbed protein, respectively. The elution percentage was defined as

$$\text{elution percentage} = 100[(\text{amount eluted})/(\text{amount adsorbed} - \text{amount washed})] \quad (4)$$

2.5. Evaluation of binding capacity of membrane

The corresponding binding capacity of the porous ion-exchange membrane, q, calculated by integrating the breakthrough curve, is as follows:

$$q = \int_0^{V_s} (C_0 - C)\, dV/M_3 \quad (5)$$

where C_0 and C are the protein concentrations of the feed and effluent, respectively, V is the effluent volume and V_s is the effluent volume when C reaches C_0.

The theoretical binding capacity q_t for protein adsorbed as a monolayer on the membrane is

$$q_t = a_v M_{rp}/(aN_A) \quad (6)$$

where a_v is the specific surface area of the membrane, a is the cross-sectional area occupied by a protein molecule with an end-on orientation, N_A is Avogadro's number and M_{rp} is the molecular mass of the protein.

3. Results

3.1. Preparation of diethylamino-containing fibre

A polymer chain containing epoxy groups was grafted on to a porous polyethylene hollow-fibre membrane by radiation-induced graft polymerization of GMA. Subsequently, part of the epoxy groups produced were converted into DEA groups and the remaining epoxy groups were converted into EA groups. The resultant hollow fibre is referred to as a DEA(X)-EA-T fibre, where X is the DEA group density and T stands for tubular shape. The DEA group density ranged from 0 to 2.9 mol per kilogram of the product by varying the reaction time. A uniform distribution of chloride ions captured by the

DEA group throughout the hollow fibre was observed using an electron probe X-ray microanalyser. The specific surface area of the DEA-EA-T fibre was constant at 5.0 m^2/g irrespective of the DEA group density. After grafting and subsequent chemical modification, the hollow fibre extended from 10 to 13 cm in length due to swelling. The DEA(2.9)-EA-T fibre swelled 3.2 times compared with the starting hollow fibre; therefore, the pore structure observed by scanning electron microscopy (SEM) was maintained.

3.2. Protein adsorption and elution during permeation

Bovine serum albumin (BSA) solution was forced to permeate through the DEA(1.7)-EA-T fibre at different flow-rates. Fig. 3 shows the breakthrough curve, i.e., BSA concentration as a function of effluent volume. The flow rate, ranging from 20 to 100 ml/h, corresponded to mean residence time, ranging from 165 to 33 s. The breakthrough curves overlapped irrespective of the residence time. Also, a sharp peak of the elution curve was obtained irrespective of the flow-rate.

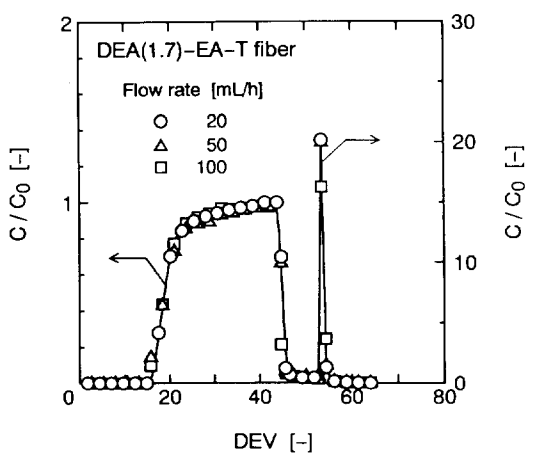

Fig. 3. Breakthrough and elution curves for the porous hollow-fibre membrane containing diethylamino (DEA) groups at different flow-rates: ○ = 20; △ = 50; □ = 100 ml/h. The ordinate C/C_0 is the concentration of the effluent relative to the feed; the abscissa is dimensionless effluent volume (DEV), defined as the effluent volume divided by the membrane volume.

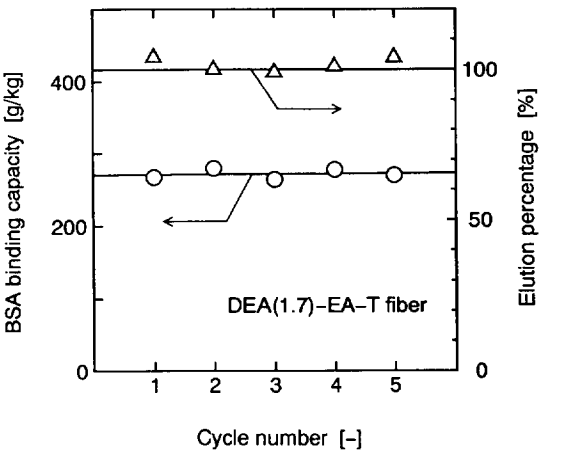

Fig. 4. Amount of BSA adsorbed and elution percentage vs. number of repeated uses.

3.3. Dependence of binding capacity and elution percentage on repeated use

Fig. 4 shows the amount of BSA adsorbed and the elution percentage in each cycle consisting of adsorption, washing and elution, in that sequence. BSA could be quantitatively eluted with Tris–HCl buffer solution containing 0.5 *M* NaCl. No deterioration of the physical strength or the binding capacity of the membrane was observed during five cycles of adsorption, washing and elution.

3.4. Dependence of binding capacity on DEA group density

Fig. 5 shows the binding capacity of the DEA(*X*)-EA-T fibre, q, calculated by integrating the breakthrough curves. The dashed line represents the theoretical binding capacity, q_t, for BSA adsorbed on the DEA(*X*)-EA-T fibre in a monolayer with an end-on orientation. A higher density of the DEA groups on the membrane led to a higher binding capacity for BSA. For example, the DEA(2.9)-EA-T fibre had a

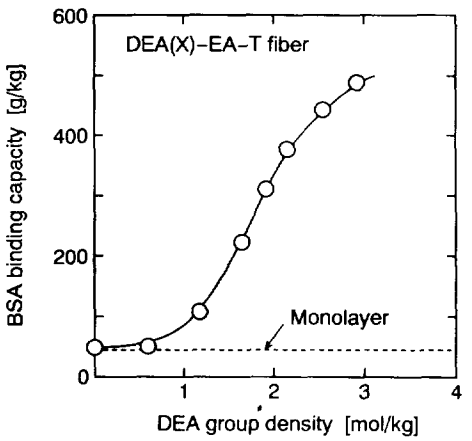

Fig. 5. BSA binding capacity as a function of DEA group density.

BSA binding capacity of 490 g/kg, which was eleven times higher than q_t.

3.5. Adsorption of various proteins

Three kinds of proteins, lactoglobulin (M_{rp} = 18 000, 3.6 × 3.6 × 3.6 nm, pI = 5.0) [12], bovine serum albumin (BSA, M_{rp} = 67 500, 4.0 × 4.0 × 11.5 nm, pI = 4.9) [13] and bovine γ-globulin (M_{rp} = 169 000, 4.4 × 4.4 × 23.5 nm, pI = 5.8–7.3) [14], were tested in the permeation mode, using the same buffer solution (pH 8). The binding capacities for the proteins bound to the DEA(1.7)-EA-T fibre are summarized in Table 1. The proteins tested were held in a tentacle-like manner by the graft chain.

4. Discussion

We succeeded in preparing a novel material in a hollow-fibre form that meets the three requirements for high-throughput processing of proteins: high rate, high capacity and high reproducibility. First, we aimed to transport the protein by convection through the pores of the hollow-fibre membrane immobilizing the ion-exchange group-containing graft chain. The protein solution was forced to permeate across the porous ion-exchange membrane. As a result, the breakthrough curve was independent of flow-rate, i.e., the residence time, because of negligible diffusional mass-transfer resistance. This means that a higher flow-rate of the protein

Table 1
Summary of properties and protein adsorption performance of tentacle-type ion exchangers

Support material			Grafting method	Monomer[a]	Protein	Monolayer adsorption (g/kg)[b]	Binding capacity (g/kg)	Ref.
Type	Shape	Structure						
Polyethylene	Hollow fibre	Porous	Radiation	GMA-DEA	Lactoglobulin	15	266	This work
					BSA	45	49–490	
					BGG	93	387	
Polyethylene	Hollow fibre	Porous	Radiation	GMA-SS	Lysozyme	18	40–200	[15]
Fractogel[c]	Bead	Non-porous	Chemical	DEAEAAm	BSA		70–140 g/l	[6]
				AAc	Haemoglobin		70–140 g/l	
				AMPS	Lysozyme		70–140 g/l	
Silica	Bead	Porous	Chemical	TMAEAAm	BSA		50	[7]

[a] DEAEAAm = Diethylaminoethylacrylamide; AAc = acrylic acid; AMPS = 2-acrylamide-2-methylpropanesulfonic acid; TMAEAAm = trimethylaminoethylacrylamide; GMA = glycidyl methacrylate; SS = sodium sulfide; DEA = diethylamine.
[b] Theoretical binding capacity for protein adsorbed as a monolayer with an end-on orientation on the ion exchanger.
[c] Copolymer of oligoethylene glycol, glycidyl methacrylate and pentaerythrol dimethacrylate.

solution through the membrane can result in a higher collection rate of the protein.

Second, the ion-exchange group-containing graft chains extended from the pore surface towards the pore interior owing to mutual electrostatic repulsion. A higher density of the ion-exchange groups was found to lead to a higher capacity for protein binding owing to the presence of three-dimensional adsorption sites for proteins. As a result, the DEA-containing membrane could hold an amount of bovine serum albumin eleven times greater than that which could be adsorbed as a monolayer on the interface.

Third, we designed the polymer chains grafted on to a porous membrane made of polyethylene in such a way that led to enhancement of the selective adsorption of the target proteins by the anion-exchange (DEA) groups and retardation of non-selective adsorption of the proteins by alcoholic hydroxyl (EA) groups. The EA group functioned as both an anion-exchange group and a hydrophilic group: the EA-containing fibre [DEA(0)-EA-T fibre], which was obtained via 90% conversion of the GMA-grafted fibre with ethanolamine, allowed monolayer BSA adsorption, as shown in the plot corresponding to a DEA group density of zero in Fig 5. As BSA could be quantitatively eluted from the DEA(0)-EA-T fibre, satisfactory quenching of the epoxy groups was realized. Quantitative elution of the protein by permeating a buffer solution containing 0.5 *M* NaCl was achieved for DEA-EA-T fibres whose DEA group density ranged up to 2.9 mol/kg. A higher rate of elution was also attained by convection of the adsorbed protein from the ion-exchange group to the bulk.

Vertical layering of the protein by the DEA-containing graft chain, i.e., tentacle binding, was confirmed for bovine γ-globulin, lactoglobulin and BSA. In a previous study [15], the combination of lysozyme and a sulfonic acid group-containing graft chain also exhibited a tentacle-like interaction. Table 1 summarizes the tentacle binding in previous studies [6,7,15]. Tentacle binding was found not to be an exceptional interaction between a protein and the ionizable graft chain.

Membrane chromatography [1] and perfusion chromatography [2] have been suggested as methods of protein processing aided by convective transport. Tentacle binding using chemically modified beads was reported by Muller [6]; however, the degree of tentacle binding has not previously been investigated as a function of the chemical structure of the graft chain. In this study, we realized simultaneously high-rate and high-capacity processing of proteins while retarding non-selective adsorption of the protein using a porous and tentacle ion-exchange membrane prepared by radiation-induced graft polymerization of the epoxy-containing monomer and subsequent modification into an anion-exchange moiety.

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