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High-performance purification of gelsolin from plasma using anion-exchange porous hollow-fiber membrane

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Abstract

Gelsolin was purified from bovine plasma using an anion-exchange porous hollow-fiber membrane. The anion-change porous hollow-fiber membrane was prepared by radiation-induced graft polymerization of an epoxy-group-containing monomer, glycidyl methacrylate, and subsequent chemical modifications. Some of the epoxy groups of the polymer chain grafted onto the pore surface were converted into diethylamino groups, and the remaining epoxy groups were converted into 2-hydroxyethylamino groups. First, a gelsolin-containing dialyzed protein solution, prepared by pretreatments of ammonium sulfate precipitation and dialysis of plasma, was forced to permeate through the pores of an anion-exchange porous hollow-fiber membrane. Various proteins including gelsolin were adsorbed onto the anion-exchange polymer brush at a high rate with negligible diffusional mass-transfer resistance. Second, adsorbed gelsolin was specifically eluted by permeating 2 mM calcium chloride. The amount of recovered gelsolin was 0.1 mg per 1 mL of plasma. Third, the remaining adsorbed proteins were quantitatively eluted with 1 M sodium chloride, leading to a constant amount of recovered gelsolin during four cycles of purification. The total time required for gelsolin purification from 30 mL of bovine plasma was 11 h, during which the time for selective adsorption of various proteins and affinity elution of gelsolin using the anion-exchange porous hollow-fiber membrane was 20 min.

Keywords: Gelsolin; Plasma; Polymer brush; Anion exchange; Purification; Membrane chromatography; Calcium ion

1. Introduction

Gelsolin (Mr, 90 kDa; p*I*, 5.8) belongs to the actin-binding proteins and controls the gel–sol transformation of actin [1]. The amount of gelsolin expressed in cells varies during canceration and differentiation of the cells. An increase in the level of expressed gelsolin suppresses apoptosis of cells; inversely, the apoptosis activates the expression of gelsolin [2].

Gelsolin is dissolved in plasma at a concentration of 0.179 mg/mL, which is equivalent to 0.2 wt.% of total serum proteins [3]. Gelsolin exhibits a strong severing activity in plasma for actin filaments originating from dead cells and binds the terminals of actin filaments to interfere with the addition of actin monomer, resulting in the prevention of the repolymerization of actin into actin filaments [4,5].

A rapid and simple method of gelsolin purification from blood was suggested by Kurokawa et al. in 1990 [6] using a bed charged with anion-exchange beads. After the loading of various proteins dissolved in plasma onto the anion-exchange beads, the specific elution of bound gelsolin was achieved by contact with a calcium ion-containing solution. However, this purification method of gelsolin using beads has a drawback in that a long processing time required for the protein solution to flow through the bed results in the unfavorable hydrolysis of gelsolin.

As a novel material capable of reducing the processing time of protein purification, porous membranes [7] and beads [8] with flow-through pores have been suggested. We

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have to date prepared ion-exchange porous membranes of a hollow-fiber form by radiation-induced graft polymerization. Ion-exchange polymer brushes, i.e., ion-exchange-group-containing polymer chains grafted onto the pore surface of a porous hollow-fiber membrane, were appended. The high-speed purification of proteins was achievable because the proteins were transported to the ion-exchange groups of the polymer brushes aided by convective flow through the pores: a negligible diffusional mass-transfer resistance of the proteins to the ion-exchange groups was demonstrated under instantaneous intrinsic ion-exchange kinetics [9,10]. Furthermore, since the ion-exchange polymer brushes capture the proteins in multilayers [11], the ion-exchange porous hollow-fiber membrane enabled a higher degree of enrichment of the proteins compared with conventional beds charged with ion-exchange beads. For example, urease is bound at the adsorbed urease level of 1.6 g per gram of anion-exchange porous hollow-fiber membrane [12].

The ion-exchange porous hollow-fiber membranes have been applied to the enrichment of model proteins to clarify the behavior of polymer brushes. Practical biological fluids such as plasma and broth have not yet been adopted as feed solutions for the ion-exchange porous hollow-fiber membranes. The objective of our study was to demonstrate the purification method of gelsolin from plasma using an anion-exchange porous hollow-fiber membrane.

2. Experimental

2.1. Materials

A porous hollow-fiber membrane used for the microfiltration of microorganisms and colloids was supplied by Asahi Kasei Chemicals Co., Japan. This hollow fiber made of polyethylene had inner and outer diameters of 2 and 3 mm, respectively, with an average pore diameter of 0.36 μm and a porosity of 70%. Glycidyl methacrylate (GMA, CH_2=$CCH_3COOCH_2CHOCH_2$) was purchased from Tokyo Kasei Co. and used without further purification.

Bovine blood was acquired from a local slaughterhouse. Four buffers were prepared during a series of purification procedures, namely, adsorption, washing, and elution, as listed in Table 1. All buffers contained 0.03% sodium azide and 1 mM dithiothreitol (DTT). Buffer B for washing contains ethylene glycol bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) to prevent the binding of calcium ions to gelsolin. Other reagents were of analytical grade or higher.

2.2. Preparation of anion-exchange porous hollow-fiber membrane

The preparation scheme for an anion-exchange porous hollow-fiber membrane consists of four steps: first, the starting porous hollow-fiber membrane was irradiated with an electron beam using a cascade-type accelerator to produce radicals [13]. Second, the electron-beam-irradiated porous hollow-fiber membrane was immersed in 10% (v/v) GMA/methanol solution at 313 K for 14 min to generate the graft chain. The degree of GMA grafting, defined as a percent weight increase of the hollow fiber, was set at 180%. The resultant GMA-grafted porous hollow-fiber membrane is referred to as a GMA fiber. Third, the GMA fiber was immersed in 50% (v/v) diethylamine (DEA)/water solution at 303 K for 90 min. The molar conversion of the epoxy groups of the graft chain into diethylamino groups ($-N(CH_2CH_3)_2$) was set at 55%. Finally, to reduce the nonselective adsorption of proteins to the polymer brush [11], the remaining epoxy groups were reacted with ethanolamine (EA) to afford 2-hydroxyethylamino groups ($-NHCH_2CH_2OH$). The resultant anion-exchange porous hollow-fiber membrane is referred to as a DEA-EA fiber. Properties of the DEA-EA fiber are summarized in Table 2.

2.3. Pretreatment of bovine blood

The purification process for gelsolin from bovine blood is shown in Fig. 1. Pretreatment procedures of bovine blood were according to Kurokawa et al. [6]. The pretreatment removed approximately 80% of total proteins in plasma. Briefly, bovine blood was left at ambient temperature for 1 h to clot. Plasma was obtained by centrifugation of blood operated at 9000 rpm at 288 K for 15 min. Tris–HCl concentration of 30 mL of plasma was adjusted to 50 mM (pH 8.0). The plasma was fractionated between 35 and 50% ammonium sulfate saturation at pH 8.0.

The precipitate was dissolved in 1.2 mL of buffer A and the resultant solution was dialyzed against 300 mL of

Table 1
Composition of buffers used in gelsolin purification

	Adsorption of various proteins buffer A	Washing buffer B	Elution of gelsolin buffer C	Elution of remaining proteins buffer D
NaCl (mM)	45	30	30	1000
Tris–HCl (mM)	25	25	25	25
EGTA (mM)	1	0.1	–	1
$CaCl_2$ (mM)		–	2	–
NaN_3 (%)		0.03		
DTT (mM)		1		
pH		8		

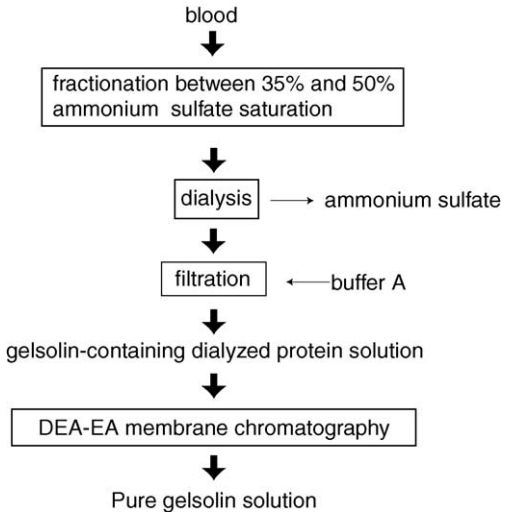

Fig. 1. Purification process for gelsolin from bovine blood.

Table 2
Properties of anion-exchange porous hollow-fiber membrane and its performance of gelsolin purification

Shape	Hollow-fiber
Size (cm)	Inner diameter: 0.26, outer diameter: 0.39, length: 5.0
Membrane volume excluding the lumen of the DEA-EA fiber (cm^3)	0.33
Functional group	Diethylamino group
Density of anion-exchange group ($mmol/cm^3$ DEA-EA fiber)	1.1
Permeation rate of the solution (mL/h)	15–120
Space velocity (h^{-1})	45–360
Amount of plasma (mL)	30
Amount of recovered gelsolin (mg)	2.9
Gelsolin binding capacity (mg/cm^3-DEA-EA fiber)	8.7
Time required for gelsolin purification using the DEA-EA fiber (min)	20

buffer A with three changes over 8 h. The semipermeable membrane for dialysis with a molecular cutoff of 14 kDa, supplied by Sanko Junyaku Co., Japan, had an effective area of 9.4 cm^2. The dialyzate (2.9 mL) was filtered through a membrane filter with a pore size of 0.45 μm (ADVANTEC DISMIC-13CP) and diluted tenfold with buffer A. The time required for the pretreatment of blood was 11 h. The resultant protein solution containing gelsolin is referred to as a gelsolin-containing dialyzed protein solution.

2.4. Affinity elution of gelsolin

Three steps of the purification of gelsolin from bovine plasma by the anion-exchange polymer brushes immobilized onto the pore surface are shown schematically in Fig. 2: (1) ion-exchange adsorption of various proteins, (2) affinity or specific elution of gelsolin, and (3) elution of remaining proteins.

A single DEA-EA fiber with an effective length of 5 cm was positioned in a U-shaped configuration with one end of the hollow fiber connected to a syringe pump and the other end sealed in a dead end. First, the gelsolin-containing dialyzed protein solution as a feed solution was forced to permeate through the pores of the DEA-EA fiber radially outward from the inside surface of the hollow fiber to the outside surface. The permeation rate of the solution through the DEA-EA fiber with a membrane volume of 0.33 cm^3, excluding the lumen, ranged from 15 to 120 mL/h; therefore, the space velocity of the solution, defined below, ranged from 45 to 360 h^{-1}.

$$\text{Space velocity, SV } (h^{-1}) = (\text{permeation rate of the solution}) / (\text{membrane volume excluding the lumen of the DEA-EA fiber}) \quad (1)$$

The effluent penetrating the outside surface of the hollow fiber was continuously collected in vials. The UV absorbance of

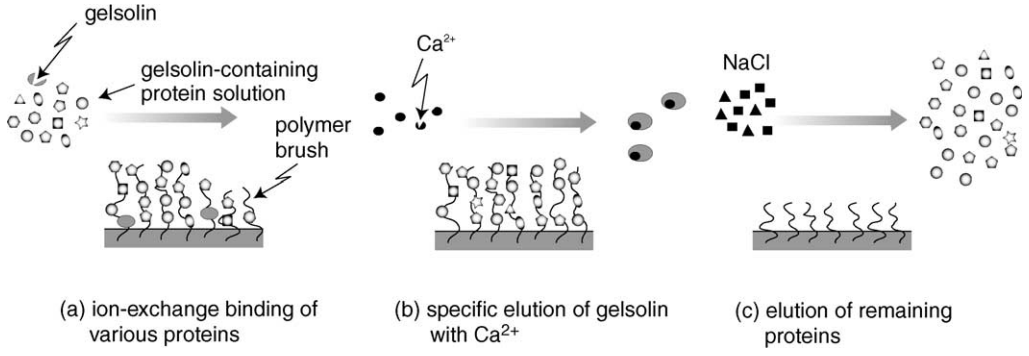

Fig. 2. Schematic illustration of purification procedures.

each vial at 280 nm was measured and converted to the concentration equivalent of globulin according to the Bradford method.

The equilibrium binding capacity (EBC), that is, the amount of protein adsorbed in equilibrium with the feed, was evaluated using

$$\mathrm{EBC\,(mg/g)} = \int_0^{Ve} (C_0 - C)\,\mathrm{d}V/W \qquad (2)$$

where C and C_0 are the protein concentrations of the effluent and feed, respectively. The terms V, V_e, and W are the effluent volume, the effluent volume when C reached C_0, and the weight of the DEA-EA fiber, respectively.

Second, after the UV absorbance of the effluent reached that of the feed, the feed solution was switched to buffer B to wash the pores. Subsequently, buffer C containing 2 mM $CaCl_2$ was permeated through the pores of the DEA-EA fiber to specifically elute gelsolin adsorbed by the anion-exchange polymer brush. The gelsolin concentration was determined in terms of globulin-equivalent concentration. Finally, the remaining proteins on the DEA-EA fiber were eluted by permeating 1 M NaCl across the hollow fiber. Elution percentage was defined as

$$\text{Elution percentage}\ (\%) = 100\frac{(\text{proteins eluted})}{(\text{proteins adsorbed})} \qquad (3)$$

The purification procedures for gelsolin were repeated using an identical DEA-EA fiber for newly pretreated plasma.

Throughout a series of purification procedures including adsorption and two-stage elution, the protein in each fraction was analyzed using Coomassie brilliant blue stained SDS-PAGE according to Laemmli [14].

3. Results and discussion

3.1. Protein adsorption onto anion-exchange porous membranes

Breakthrough curves of the DEA-EA fiber for the feed are shown in Fig. 3 as a function of the space velocity defined by Eq. (1). In this figure, the ordinate is the UV absorbance of the effluent at 280 nm relative to the feed, whereas the abscissa is a dimensionless effluent volume defined by dividing the effluent volume by the membrane volume excluding the lumen. The breakthrough curves overlapped irrespective of the space velocity. This demonstrates an advantage of the porous membrane over the bead-packed bed in that the higher space velocity of the protein solution, the higher adsorption rate of the proteins. No dependency of the breakthrough curves on the space velocity can be explained by the fact that the diffusional mass-transfer resistance of the proteins to the diethylamino groups is negligible in the overall protein binding process: the time required for the diffusion of proteins into the pore interior is negligibly shorter than the residence time of the proteins solution across the membrane thickness. This favorable characteristic was observed in the binding process of a model protein using modified porous hollow-fiber membranes in the permeation mode [10].

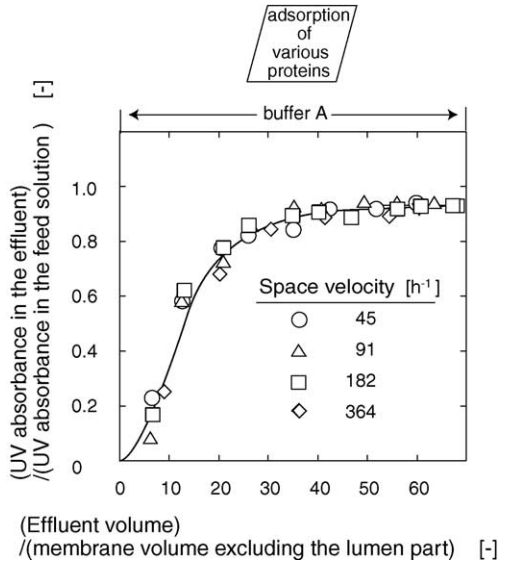

Fig. 3. Breakthrough curves as a function of space velocity of gelsolin-containing dialyzed protein solution.

3.2. Affinity elution of adsorbed gelsolin with calcium ion

Gelsolin adsorbed onto the DEA-EA fiber was specifically eluted by permeating 2 mM $CaCl_2$ through the pores, followed by the elution of remaining proteins with 1 M NaCl. Breakthrough and elution curves throughout the purification procedure are shown in Fig. 4. The permeation of two subsequent eluents produced two peaks.

SDS-PAGE lanes of the feed and effluents during various procedures are shown in Fig. 5. The symbols c to g indicate the

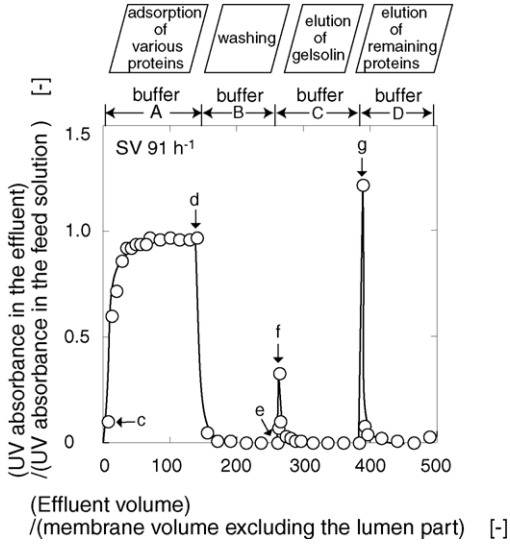

Fig. 4. Breakthrough and elution curves using anion-exchange porous hollow-fiber membrane.

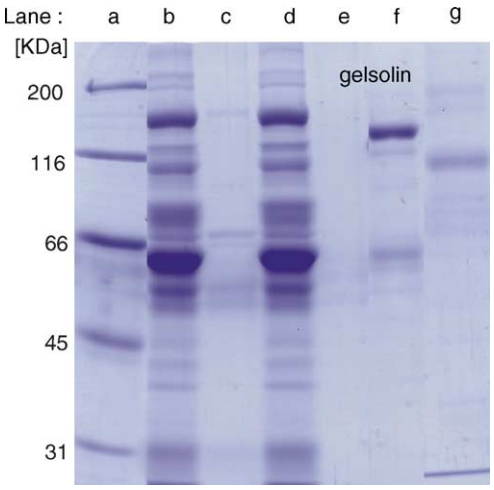

Fig. 5. SDS-PAGE analysis throughout the purification procedures for gelsolin. Lane a is molecular mass standard. Lane b corresponds to gelsolin-containing dialyzed protein solution. Lanes c–g correspond to the points identified in Fig. 4 by arrowheads.

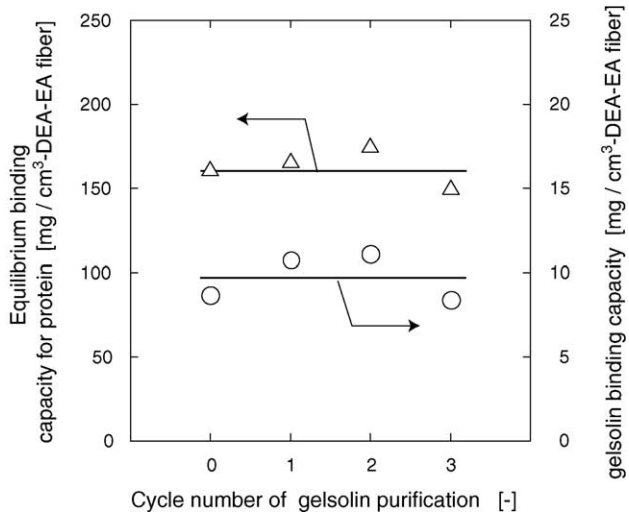

Fig. 6. Repeated use of the anion-exchange porous hollow-fiber membrane for gelsolin purification.

points identified in Fig. 4 by arrowheads; lane a is the molecular mass standard. Lane c corresponding to the first fraction from the adsorption procedure had a very faint band compared with lane b corresponding to the gelsolin-containing dialyzed protein solution as a feed solution. Most of the proteins were adsorbed by the anion-exchange polymer brush. Lane d agreed well with lane b because the adsorption system reached an equilibrium in the permeation mode. The absence of a band in lane e corresponding to the washing procedure is indicative of a negligible release of adsorbed proteins from the anion-exchange polymer brush. Lane f corresponding to the first peak obtained by the elution with 2 mM $CaCl_2$ has bands originating from a gelsolin–calcium ion complex and its hydrolyzed fractions. The observed band corresponding to 130 kDa, obtained by SDS-PAGE, is ascribed to the formation of a complex of gelsolin (Mr, 90 kDa) with actin (Mr, 40 kDa) [15]. Lane g corresponding to the second peak is difficult to analyze because the effluent was diluted 20-fold with buffer B to lower its ionic strength before SDS-PAGE.

3.3. Repeated use of the DEA-EA fiber for gelsolin purification

After affinity elution of gelsolin with calcium ion, the remaining proteins captured by the anion-exchange polymer brush were eluted with 1 M NaCl. The purification procedures of gelsolin were repeated using an identical DEA-EA fiber. The amounts of gelsolin and various proteins are shown in Fig. 6 as a function of the cycle number based on the repeated use of the DEA-EA fiber. The elution percentage defined by Eq. (3) remained 100%; all proteins adsorbed onto the hollow fiber were eluted quantitatively. Both amounts were constant during three cycles. This demonstrates that the DEA group as an anion-exchange group and the adjacent EA group as a hydrophilic group retain their respective roles: the former captures proteins selectively and the latter prevents the nonselective adsorption of proteins. The reduction of the nonselective adsorption of the proteins by the EA group was experimentally verified using various solutions of proteins such as β–lactoglubulin, bovine serum albumin, and urease [11].

3.4. Performance of gelsolin purification using the DEA-EA fiber

The results of the purification method suggested in this study are listed in Table 2 along with the properties of the anion-exchange porous hollow-fiber membrane (the DEA-EA fiber). The amount of recovered gelsolin using the DEA-EA fiber was 0.097 mg per 1 mL of plasma. The recovery was 54% on the assumption that the initial concentration of gelsolin in the bovine plasma is 0.179 mg/mL. The incomplete recovery can be explained as being due to the following two reasons: loss of gelsolin during the pretreatment of blood and partial elution of gelsolin with buffer C. The time required for gelsolin purification based on anion-exchange interaction and subsequent affinity elution using the DEA-EA fiber was 20 min.

4. Conclusion

A porous hollow-fiber membrane, the pore surface of which was modified with anion-exchange polymer brushes, was prepared by radiation-induced graft polymerization of an epoxy-group-containing monomer and subsequent introduction of diethylamino groups as anion-exchange groups into the polymer brush. Gelsolin was purified from bovine plasma using an anion-exchange porous hollow-fiber membrane on the basis of the anion-exchange adsorption of various proteins and the subsequent affinity elution of gelsolin. The following conclusions were derived:

(1) Breakthrough curves obtained by permeating a gelsolin-containing dialyzed protein solution acquired from bovine plasma through the pores rimmed by anion-exchange polymer brushes overlapped irrespective of the residence time of the solution across the hollow fiber. This is indicative of an ideal purification performance with negligible diffusional mass-transfer resistance for various proteins in the solution to the anion-exchange polymer brushes.
(2) No deterioration of the membrane during the repeated adsorptions and elutions of proteins was demonstrated because the functional groups on the polymer brush, that is, diethylamino and 2-hydroxyethylamiono groups, worked well: the diethylamino groups capture various proteins by selective binding and releases gelsolin, whereas the 2-hydroxyethylamiono groups render the surface hydrophilic to prevent the nonselective adsorption of proteins.

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