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Journal of Chromatography A, 758 (1997) 209–215

JOURNAL OF CHROMATOGRAPHY A

Selection of a precursor monomer for the introduction of affinity ligands onto a porous membrane by radiation-induced graft polymerization

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Received 25 July 1995; revised 20 August 1996; accepted 21 August 1996

Abstract

A scheme for the coupling of a ligand onto a porous membrane was selected based on criteria of permeability of liquid and adsorptivity of protein. Two precursor monomers, i.e., acrylic acid (AAc) and glycidyl methacrylate (GMA), were grafted onto a porous polyethylene hollow fibre by radiation-induced graft polymerization. The carboxyl group of the AAc-grafted hollow fibre was reacted with N-hydroxysuccinimide to produce a succinimide group as an activated group. The resultant hollow fibre had a significantly lower liquid flux than the original hollow fibre, whereas the GMA-grafted hollow fibre retained its original level of liquid permeability when the epoxy group as the activated group was introduced at a sufficiently high density.

Keywords: Graft polymerization; Affinity ligands; Membranes; Hollow fibres; Stationary phases, LC; Acrylic acid; Glycidyl methacrylate; Polyethylene; Proteins

1. Introduction

Forced convection of a protein solution through a porous membrane containing an affinity ligand along the pore can minimize the diffusional path of the protein to the binding site, resulting in high-rate collection of the protein. For example, Brandt et al. [1] purified immunoglobulin and fibronectin using a porous membrane containing Protein A and gelatin, respectively, as the ligand. We have prepared affinity and ion-exchange porous membranes of a hollow-fibre form by applying radiation-induced graft polymerization (RIGP) and subsequent chemical modifications: hydrophobic amino acids [2] and immobilized metal [3] as pseudobiospecific affinity ligands, and a sulfonic acid group [4] and a diethylamino group [5] as ion-exchange groups were introduced onto the membrane. The RIGP technique enables us to attach the polymer chain onto the porous membrane uniformly at a high density across the membrane thickness.

An activated group, such as a succinimide or an epoxy group, has been used for coupling of various ligands to conventional agarose and polyvinyl al-

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PII S0021-9673(96)00737-6

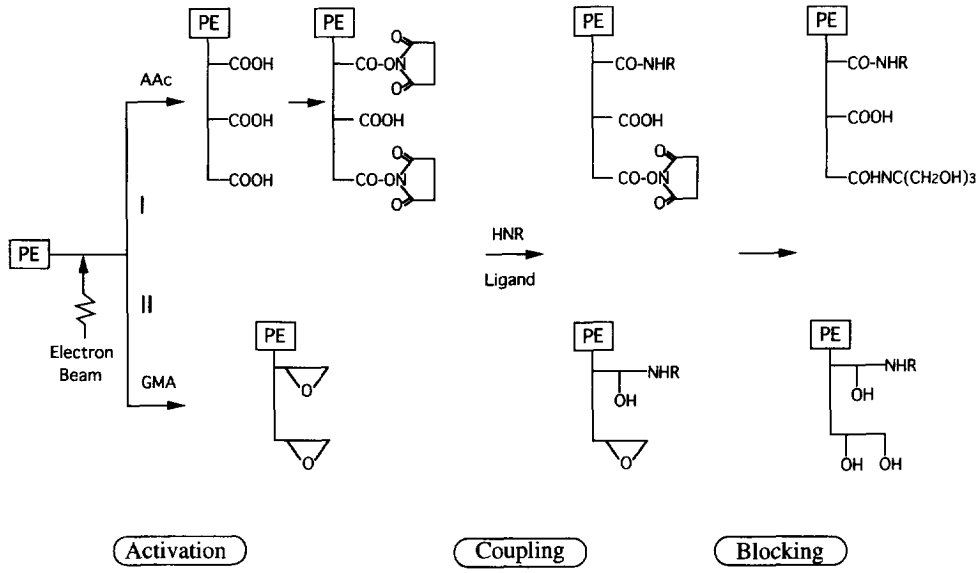

Fig. 1. Schemes for the introduction of ligand onto a polyethylene matrix.

cohol beads [6–8]. Two representative schemes of ligand introduction via an activated group are shown in Fig. 1. In order to enhance the versatility of the affinity porous membrane, the activated group should be selected based on the following three criteria: (1) permeability of the protein solution through the membrane, (2) specific binding capacity of the membrane for the target protein and (3) non-selective adsorptivity of the protein onto the membrane.

The objective of our study was to select a precursor monomer grafted by RIGP onto a porous membrane that was suitable for the subsequent introduction of the affinity ligands.

2. Experimental

2.1. Materials

Two kinds of porous polyethylene (PE) hollow fibres (Asahi Chemical Industry, Tokyo, Japan) were used as trunk polymers for grafting. Properties of these hollow fibres are summarized in Table 1. Technical-grade acrylic acid (AAc, CH_2=CHCOOH) and glycidyl methacrylate (GMA, CH_2=$CCH_3COOCH_2CHOCH_2$) were purchased from Kanto Tokyo, Japan and Tokyo Kasei, Tokyo, Japan, respectively. Soybean trypsin inhibitor (STI), bovine serum albumin (BSA), and bovine γ-globulin (BGG) were purchased from Sigma (St. Louis, MO, USA; STI, No. T-8253, Type III; BSA, No. A-7030, essentially fatty-acid and γ-globulin free; BGG, No. G-5009, Cohn Fraction II, III). Other reagents were of reagent grade.

2.2. Introduction of a succinimide group onto a porous hollow fibre

Ligand coupling via a succinimide group is shown in scheme I of Fig. 1. First, the PE hollow fibre was irradiated by an electron beam from a cascade-type accelerator (Dynamitron, Model IEA 3000-25-2; Radiation Dynamics, New York, NY, USA) at a total dose of 200 kGy in a nitrogen atmosphere at ambient temperature. Second, the irradiated hollow fibre was placed in contact with the AAc, which had been

Table 1
Properties of original hollow fibres

	AAc grafting	GMA grafting
Inner diameter (mm)	0.6	1.9
Outer diameter (mm)	1.2	3.2
Porosity (%)	67	70
Pore diameter (μm)	0.20	0.20

deaerated by repeated freezing and thawing in the vapor phase to expel any dissolved oxygen. The degree of grafting (dg) and the density of the carboxyl group per g of trunk polymer were defined as

$$\mathrm{dg}(\%) = 100\,(W_1 - W_0)/W_0 \quad (1)$$

density of carboxyl group (mol/g of trunk polymer)

$$= (W_1 - W_0)/72/W_0 \quad (2)$$

where W_0 and W_1 are the masses of the original and AAc-grafted hollow fibres, respectively, and 72 is the molecular mass of AAc. The degree of AAc grafting ranged from 20 to 80%. The resultant hollow fibre is referred to as an AAc fibre. Third, the AAc fibre was immersed in a 0.15 *M* N-hydroxysuccinimide (NHS) aqueous solution with an equimolar amount of water-soluble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride] at 303 K. The initial molar ratio of NHS to the carboxyl group of the AAc fibre was set to 15–30. The reaction time ranged up to 6 h. The resultant hollow fibre is referred to as an AAc–Su fibre. After introduction of the succinimide group, the hollow fibre was dried under reduced pressure and weighed. The density of the succinimide group per g of trunk polymer was calculated as

density of succinimide group (mol/g of trunk polymer) $= (W_2 - W_1)/97/W_0$ (3)

where W_2 is the mass of the AAc–Su fibre and 97 is the molecular mass of NHS.

2.3. Introduction of an epoxy group onto a porous hollow fibre

The coupling scheme of the ligand via an epoxy group is shown in scheme II of Fig. 1. An epoxy-group-containing vinyl monomer, GMA, was grafted onto the porous PE hollow fibre in a liquid phase; the irradiated hollow fibre was immersed in 10% (v/v) GMA in a methanol solution that had been previously deaerated by bubbling with nitrogen at 313 K. The degree of GMA grafting ranged from 20 to 250%, with varying reaction times. The resultant hollow fibre is referred to as a GMA fibre. The density of the epoxy group per g of trunk polymer was calculated as

density of epoxy group (mol/g of trunk polymer)

$$= (W_1 - W_0)/142/W_0 \quad (4)$$

where W_1 is the weight of the GMA fibre and 142 is the molecular mass of GMA.

2.4. Coupling of ligand with a succinimide group

STI, as an affinity ligand, was coupled with the AAc–Su fibre. The fibre was immersed in a 0.05 g of STI/l carbonate buffer (0.012 *M* $NaHCO_3$ + 0.15 *M* NaCl, pH 8) solution at 277 K. The coupling percentage (X_c) was defined as

$X_c(\%) = 100$ (moles of STI coupled)/

(moles of succinimide group of the AAc–Su fibre) (5)

where the number of moles of STI coupled was calculated from the decrease in the STI concentration of the solution determined by liquid chromatography (Asahipak GS-520H column, Asahi Chemical Industry). Subsequently, the residual succinimide groups were blocked with a Tris–HCl buffer solution (pH 8.3) at ambient temperature.

2.5. Coupling of ligand with an epoxy group

Phenylalanine (Phe) as a pseudobiospecific affinity ligand was reacted with the GMA fibre. The hollow fibre was immersed in a 0.45 *M* Phe aqueous solution at 353 K. The residual epoxy groups were quantitatively converted to diol groups by acid hydrolysis [9]. The resultant pseudobiospecific affinity hollow fibre is referred to as a Phe fibre. The X_c was defined as

$X_c(\%) = 100$ (moles of Phe coupled)/

(moles of epoxy group of the GMA fibre)

$$= [(W_2 - W_1)/165]/[(W_1 - W_0)/142] \quad (6)$$

where W_2 is the mass of the Phe fibre and 165 is the molecular mass of Phe.

2.6. Liquid permeability of the porous membrane

A hollow-fibre membrane about 10 cm long was positioned in an I- or U-shaped configuration. An experimental apparatus for measurement of liquid permeability of the hollow fibre has been described in our previous publication [10]. An aqueous NaCl solution was forced to permeate radially outward at a constant permeation pressure of 0.1 MPa. The concentration of NaCl ranged from 0.1 to 2 *M* (pH 5.1). The flow-rate of the effluent penetrating the outside surface of the hollow fibre was measured, and the flux was calculated by dividing the flow-rate by the inside surface area of the hollow fibre. The measurement was performed at 303 K.

2.7. Protein adsorption and elution during permeation

A protein adsorption and elution experiment was performed using the affinity hollow-fibre membrane that was about 10 cm long in a dead-end mode with respect to the lumen. A binary protein solution containing 1 g/l BSA and 1g/l BGG was permeated through the affinity porous hollow-fibre membrane from the inside to the outside at a constant flow-rate of 30 ml/h using an infusion syringe pump (ATOM 235, ATOM, Tokyo, Japan). The proteins were dissolved in a 0.01 *M* Tris–HCl buffer solution containing 3.3 *M* NaCl (pH 8). The protein concentration of the effluent was continuously determined by liquid chromatography. After equilibration, the buffer solution and eluent were serially permeated through the hollow fibre to wash the pores and elute the proteins, respectively. The NaCl-free buffer solution was used as an eluent.

3. Results and discussion

3.1. Coupling of ligand with an activated group

STI and Phe were coupled with the succinimide and epoxy groups, respectively, on the polymer chain grafted onto the porous PE membrane of a hollow-fibre form. About 13% of the carboxyl groups of the AAc-grafted hollow fibre (AAc fibre) with a dg of 22% were converted to succinimide groups after 6 h,

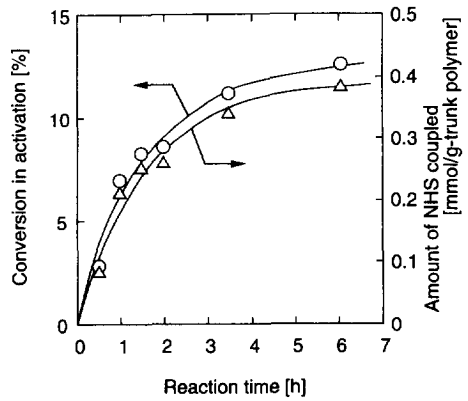

Fig. 2. Conversion of carboxyl groups of AAc-grafted hollow fibre to succinimide groups.

as shown in Fig. 2. The coupling percentage of the activated hollow fibre as a function of the reaction time with STI is shown in Fig. 3a. STI was coupled with 5.6% of the succinimide groups after 15 h and the resulting ligand density was 2.1×10^{-2} mmol per g of trunk polymer. The density of the residual carboxyl groups amounted to 2.7 mmol per g of trunk polymer, which is almost equivalent of that of a conventional weakly acidic cation-exchange bead. This low conversion of carboxyl group to affinity ligand induces the protein binding based on ion-exchange interaction as well as affinity interaction, which is unfavorable to specific binding of the target protein.

The hydrophobic amino acid, Phe, was coupled with the epoxy group of the GMA-grafted hollow fibre (GMA fibre) with a dg of 130% to yield a final coupling percentage of 14%, i.e., a ligand density of 1.3 mmol per g of trunk polymer, as shown in Fig. 3b. Since the protein binding capacity of affinity porous membranes is determined from the surface area of pores in the membrane [11], the resultant ligand density will be sufficient for protein binding.

3.2. Liquid permeability of a modified porous hollow fibre

Negligible mass-transfer resistance of the protein to the ligand and ion-exchange groups immobilized on the pore surface of the porous membrane was observed in pseudoaffinity and ion-exchange chromatographic modes [12,13]. In contrast, affinity

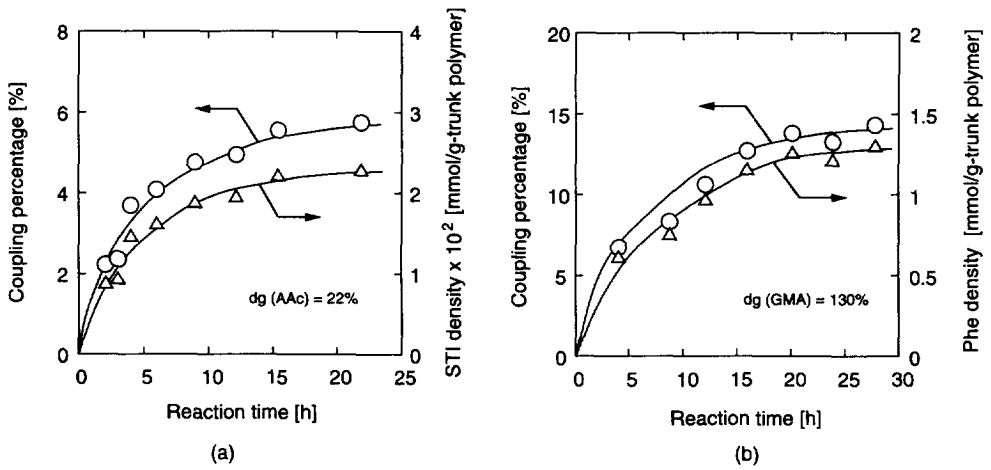

Fig. 3. Time courses of coupling percentage: (a) STI ligand via a succinimide group; (b) Phe ligand via an epoxy group.

porous membranes involving antigen–antibody pairs, e.g., a pair of recombinant human interferon-α2a and its monoclonal antibody [14], diminish the advantage because of the pair's slow intrinsic interaction kinetics compared to the residence time of the solution through the porous membrane. For both cases, liquid permeability of the modified porous membrane should be evaluated for membrane chromatography.

When the ligand was introduced at a density yielding a low coupling percentage, e.g., 3% in the case of Phe coupled with the GMA fibre, the liquid permeability of the resultant hollow fibre was mainly determined at the stage of graft polymerization of the precursor vinyl monomers, such as AAc and GMA. The flux of NaCl solution through the AAc and GMA fibres with different degrees of grafting is shown in Fig. 4a–b, respectively. In these figures, the ordinate F/F_0 is the flux ratio of the modified membrane to the trunk polymer. The AAc fibre exhibited a significantly low flux of 0.1 *M* NaCl solution over dg = 30%, because a high density of ionizable groups, i.e., carboxyl groups, on the poly-

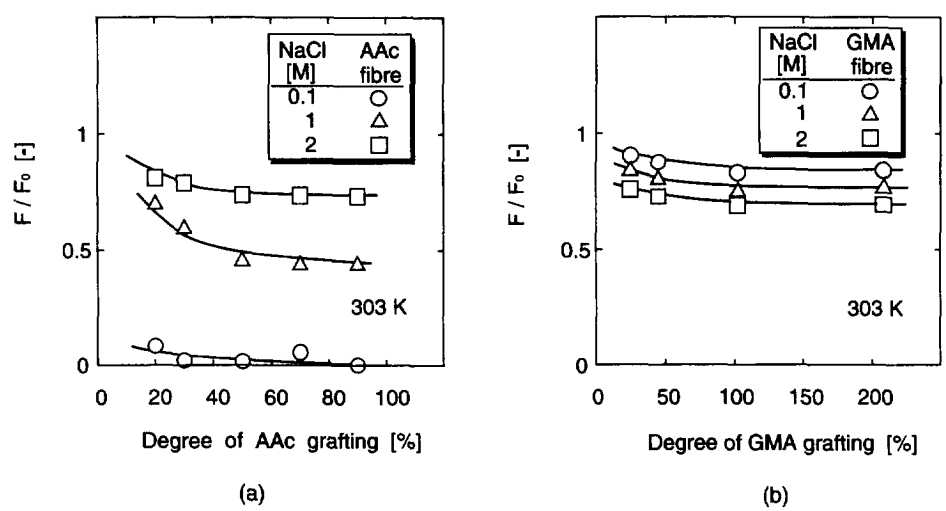

Fig. 4. Flux ratio of modified hollow fibre to original hollow fibre for NaCl solution: (a) AAc-grafted hollow fibre; (b) GMA-grafted hollow fibre.

AAc graft chains induced a high degree of extension of the graft chains into the pore interior in liquid due to mutual repulsion of the graft chains. At present, the isolation of poly-AAc or poly-GMA graft chains from PE is difficult because of the high chemical stability of PE. Yamagishi et al. [15] isolated the polymethyl methacrylate graft chain from the porous cellulose triacetate membrane and found that the length of the graft chain is of the order of sub-μm; therefore, the extension of the graft chain will cause a significant decrease in the flux of the modified hollow fibre. An increase in the ionic strength increases the charge shielding of the ionizable polymer chains; a high NaCl concentration restricts the extension of poly-AAc graft chains, resulting in a higher degree of permeability of 1 and 2 *M* NaCl solutions through the pores of the AAc fibre. On the other hand, the GMA fibre showed almost constant permeability, irrespective of the ionic strength, because the epoxy groups on the poly-GMA graft chain are electrostatically neutral.

3.3. Chromatography of proteins using a porous membrane

Coupling of the ligands via the epoxy group of the GMA fibre was advantageous over coupling via the succinimide group of the AAc–Su fibre because the resultant membrane in the former case showed higher permeability of the protein solution and eluent than those in the latter case. Moreover, blocking of the residual epoxy groups, i.e., converting them to diol groups by addition of water, can render the pore surface hydrophilic, to reduce the non-selective adsorption of the protein [16,17]. Kim et al. [17] reported that satisfactory hydrophilization occured when diol groups were introduced to the pore surfaces, which resulted in an elution percentage of 100% for BSA and BGG upon increasing the NaCl concentration in the buffer.

A chromatogram for a binary system of BSA and BGG during adsorption, washing and elution using the Phe fibre (dg = 110%, coupling percentage = 14%) is shown in Fig. 5. The Phe fibre had higher selectivity for BGG than for BSA; almost all of the BSA was eluted simply by washing with the Tris–HCl buffer containing 3.3 *M* NaCl. The equilibrium binding capacity was 54 mg of BGG per g of Phe

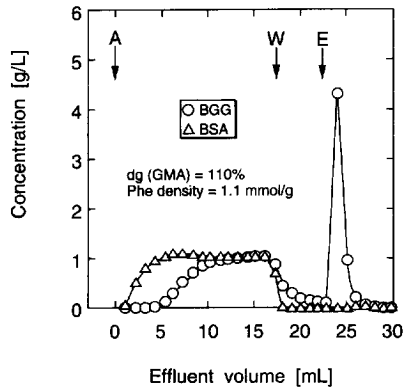

Fig. 5. Concentration changes of BSA and BGG during adsorption (A), washing (W) and elution (E).

fibre. The adsorbed BGG could be quantitatively eluted by permeating the NaCl-free buffer through the Phe fibre. This is indicative of a negligible amount of the protein being adsorbed non-selectively.

4. Conclusions

We suggested two preparation schemes for the introduction of activated groups, i.e., succinimide and epoxy groups, onto a porous polyethylene hollow fibre by applying radiation-induced graft polymerization of acrylic acid and glycidyl methacrylate, respectively, and subsequent chemical modifications. First, the succinimide group was attached via a reaction of the carboxyl group of the AAc-grafted membrane with N-hydroxysuccinimide. The resultant membrane exhibited lower liquid permeability after the introduction of STI because the residual carboxyl groups on the graft chains induced a higher degree of extension of the graft chains. Second, the epoxy group was introduced by graft polymerization of the epoxy-group-containing vinyl monomer (GMA). Liquid permeability of the resultant membrane was retained at the original level when phenyalanine was introduced at a sufficiently high ligand density. The poly-GMA chain was found to be suitable for the introduction of the affinity ligands along with a hydrophilic group onto a porous membrane because the membrane showed higher permeability and lower non-selective adsorptivity for the protein solution.

5. Symbols and abbreviations

AAc	acrylic acid
BGG	bovine γ-globulin
BSA	bovine serum albumin
dg	degree of AAc or GMA grafting defined by Eq. (1) (%)
F	permeation flux of modified hollow fibre (m/s)
F_0	permeation flux of original hollow fibre (m/s)
GMA	glycidyl methacrylate
NHS	N-hydroxysuccinimide
Phe	phenylalanine
STI	soybean trypsin inhibitor
Su	succinimide
W_0	mass of original hollow fibre (g)
W_1	mass of AAc- or GMA-grafted fibre (g)
W_2	mass of AAc-Su or Phe fibre (g)
X_c	coupling percentage of affinity ligand, defined by Eqs. (5,6) (%)

Acknowledgments

The authors thank Yoshizumi Inai of the Industrial Membrane Division of Asahi Chemical Industry Co., Ltd., Japan, for providing the original hollow fibre.

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