Template Imprinting Amphoteric Polymer for the Recognition of Proteins

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ABSTRACT: A selective recognizing of proteins with amphoteric polymer is essential for application in protein separations. An amphoteric polymer was synthesized by copolymerization of the two functional monomers with the crosslinker in the presence of the template proteins. After extraction of the template protein, the polymer was applied as chromatographic stationary phase, showing high resolution capacity. The molecular imprinting technique allows the formation of specific recognition sites in amphoteric polymers through the use of template protein. Protein is preferentially bound to a polymer made in the presence of the same protein. The recognition ability of an amphoteric polymer is attributed to imprinted cavities that complement proteins in shape and electrostatic interactions. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 95: 358–361, 2005

Key words: molecular recognition; proteins; molecular imprinting; macroporous polymers

INTRODUCTION

The principle in selectively recognizing proteins is built on an assumption that the nanocavities of a polymer complement proteins in size, shape, hydrogen bonding, and electrostatic profiles. In this respect, it is similar to molecularly imprinting techniques (MIT). Application of MIT ranges widely from polymer catalysts to sensor design,[1–4] artificial receptors/antibodies,[5–7] and HPLC stationary phases for chiral resolution.[8,9] This area of research is too large to survey here, but see Haupt,[10] Byrne et al.,[11] Peppas and Huang,[12] and Wulff[13] for recent reviews.

Although polymer-imprinted protein has been available in protein recognition,[14–18] attempts to imprint proteins have met with only limited success, especially for the imprinting of bulk polymers.

We introduced monomers containing tertiary amine and carboxyl acid groups into polymer units to create an amphoteric copolymer chain network. We selected two template proteins, bovine serum albumin (BSA) and chicken egg white lysozyme (LSZ). The amphoteric copolymer was imprinted by a protein to give the functional materials capable of recognizing this protein. The amphoteric copolymer is bound in the protein surface polar residues by multiple electrostatic interactions and hydrogen bonds, which can be useful in fabricating chromatographic stationary phases.

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EXPERIMENTAL

General procedures

N-[3-(Dimethylamino)propyl]methacrylamide was purchased from J&K Chemical Ltd. (Beijing, China). Chicken egg white lysozyme, bovine serum albumin, and other chemical reagents were obtained from Tianjin Chemical Reagent Co., Inc. (Tianjin, China). The free radial initiator, 2,2′-azobis(2-methylpropionamidine)dihydrochloride (V-50), was purified by recrystallization in water. FTIR spectra were recorded on a Nicolet 5DX spectrometer. Chromatographic experiments were carried out on a Agilent HPLC 1100LC system equipped with a model G1311A pump, a manual injector, and a model G1314A variable-wavelength absorbance detector.

Preparation of nonimprinted amphoteric polymer (1)

To a suspension of 0.3 g of 2,500 mesh of $CaCO_3$ powder (pore-forming reagent) in 30 mL of potassium phosphate buffer (pH 7.5, 0.01*M*) was added 3.41 g (20 mmol) of *N*-[3-(dimethylamino)propyl]methacrylamide, 1.72 g (20 mmol) of methacrylic acid, and 0.51 g (3 mmol) of *N*,*N*′-ethylenebisacrylamide (crosslinker). After the mixture was warmed to 60°C, 0.25 g of V-50 (initiator) was added. The suspension was bubbled with nitrogen gas until a crosslinked gel was formed. The solid was crushed and pressed through a 60 μm mesh sieve. The resulting polymer were washed five times with 250 mL of 0.1*M* hydrochloric acid to remove $CaCO_3$ and was dried under vacuum

to give 4.0 g (71%) of **1** as white powder. IR: 3,200–2,800, 1,710, 1,480, 940 cm^{-1}.

LSZ imprinting of the amphoteric polymer (2)

To a suspension of 0.15 g of LSZ and 0.3 g of 2,500 mesh of $CaCO_3$ powder in 20 mL of potassium phosphate buffer (pH 7.5, 0.01*M*) was added 3.41 g (20 mmol) of *N*-[3-(dimethylamino)propyl]methacrylamide, 1.72 g (20 mmol) of methacrylic acid, and 0.51 g (3 mmol) of *N,N'*-ethylenebisacrylamide. After the mixture was warmed to 60°C, 0.25 g of V-50 was added. The suspension was bubbled with nitrogen gas until a crosslinked gel was formed. The solid was crushed and pressed through a 60 μm mesh sieve size. The resulting polymer was washed five times with 100 mL of 0.1*M* hydrochloric acid to remove $CaCO_3$. The particles were treated for 48 h with 2.0 mg of pronase (type XXI, from *Streptomyces griseus*) in 5 mL of potassium phosphate buffer (pH 8.0, 0.1*M*) to remove partial protein.[19] The product was dried under vacuum to give 3.8 g (68%) of **2** as white powder. IR: 3,200–2,800, 1,700, 1,460, 930 cm^{-1}.

BSA imprinting of the amphoteric polymer (3)

To a reaction system was added 0.15 g of BSA as template protein. The polymer was synthesized according to the procedure described in the polymerization of **2.** The resulting polymer was dried under vacuum to give 3.7 g (65%) of **3** as white powder. IR: 3,200–2,800, 1,710, 1,470, 920 cm^{-1}.

High-performance liquid chromatography[9]

The protein-imprinted polymers were slurried in chloroform-acetone (15 : 3, v/v) and packed with acetone as solvent into stainless-steel columns at 300 bar using an air-driven fluid pump. The proteins were absorbed and extracted on the polymer (**1, 2,** and **3**) by eluting with methanol-acetic acid (8 : 1, v/v). The elution was performed at ambient temperature and was monitored spectrophotometrically at 220 nm.

RESULTS AND DISCUSSION

LSZ or BSA, as the template protein, was used to orient monomers prior to polymerization. *N*-[3-(dimethylamino)propyl]methacrylamide (MAPMAm) and methacrylic acid (MAA) are ionizable monomers, which frequently will act as acid, base, nucleophile, or electrophile units. MAPMAm is preferentially closed up to Asp and Glu residues of a protein, and MAA is preferentially closed up to Lys, Arg, Ser, and His owing to electrostatic interactions.

As shown in Figure 1, the free radical polymerization was initiated by a water-soluble initiator V-50. The recognition sites were tailor-made by the copolymerization of the monomers and crosslinker in the presence of a template protein and $CaCO_3$ powder. The template protein and $CaCO_3$ were subsequently removed from the crosslinked polymer, leaving pore canals and recognition cavities complementary to the protein in shape and in the positioning of polar groups. $CaCO_3$, as a pore-forming reagent, was removed by an acid wash to form a macroporous polymer.[20] Protein molecules were diffused through these pore canals. Using the pronase digestion,[19] the entrapped protein was decomposed and the copolymer with high load capacity was prepared.

The ground, sieved, and extracted copolymers were used as stationary phases in the high-performance liquid chromatography. Figure 2(a) shows the resolution of LSZ by nonimprinted and LSZ-imprinted MAPMAm–MAA copolymers (**1** and **2**) packed into analytical columns, respectively. LSZ is eluted at 8.3 min from polymer **1** and but the peak disappears at the same retention time for polymer **2,** indicating that LSZ preferentially bound to the copolymer made in the presence of LSZ. Figure 2(b) shows the resolution of LSZ is by **1** or **3.** BSA is eluted at 7.9 min from the two copolymers, respectively. BSA (M_r 66 kDa) is large than LSZ (M_r 14.6 kDa) in size. This indicates that LSZ cannot be bound to the BSA-imprinted cavity, although it is loaded into the cavity.

It is believed that the side chains of the protein interact via electrostatic interactions and hydrogen bonds with the positioned amino, carboxyl, and amide groups of the polymer chain. Distribution of charge and polar on protein surface is very complicated. Alkaline MAPMAm and acidic MAA of an amphoteric polymer are random in sequence. Electrostatic distribution of a cavity is adjusted according to the protein surface, so that the electrostatic information from the template protein that is essential for recognition can be transferred to the imprinted cavity.

CONCLUSION

The cooperative, multiple electrostatic interactions lead to a significantly increased whole affinity between amphoteric polymer and protein surface. Protein imprinting of an amphoteric polymer has proven to be a useful technique for the synthesis of stationary phases selective for the corresponding proteins. The technique can also be applied to other protein separations. The polymers are easily synthesized and possess excellent mechanical and chemical stability.

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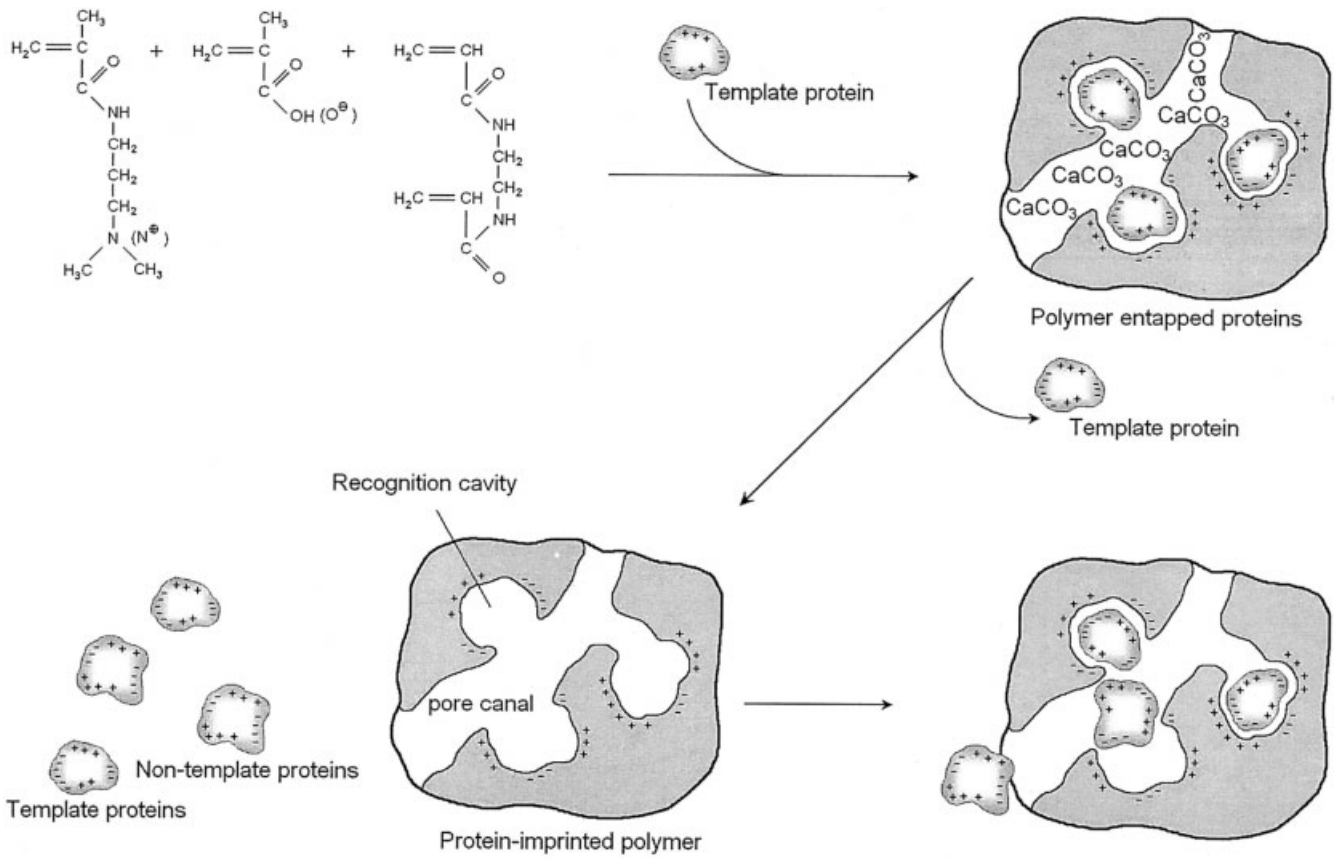

Figure 1 Protocol for template imprinting of protein with amphoteric polymer.

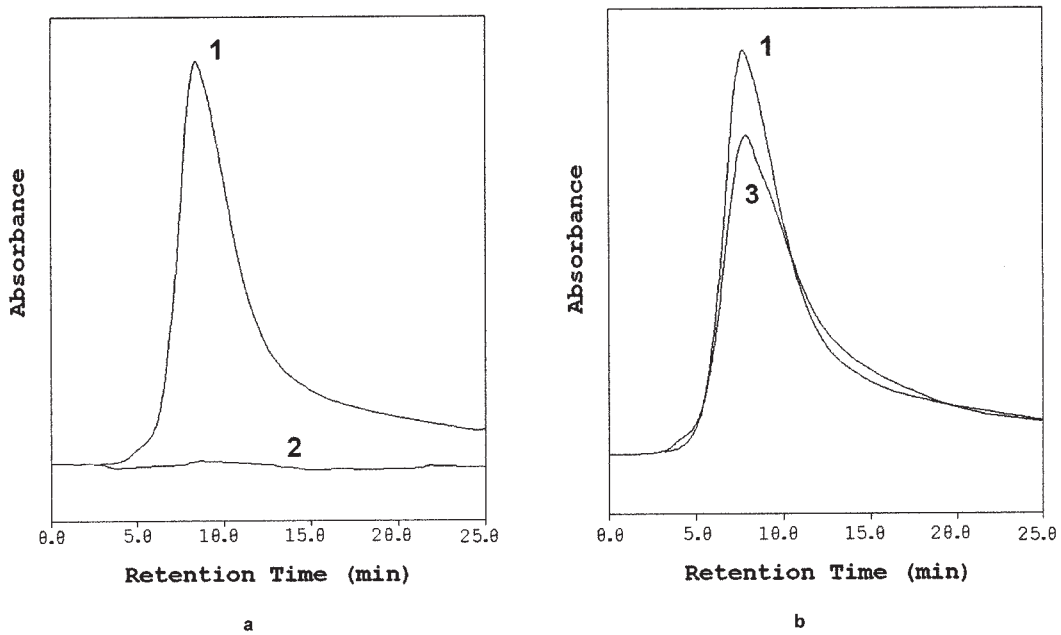

Figure 2. Selective recognition of protein by high performance liquid chromatography. **a.** Resolution of protein LSZ on a non-imprinted and LSZ-imprinted MAPMAm-MAA coplymer (**1** and **2**) packed into an HPLC column (150×4.6 mm), respectively. **b.** Resolution of protein LSZ on a non-imprinted and BSA-imprinted polymer (**1** and **3**) packed into the column, respectively.

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