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RESEARCH PAPER

Extensive Imprinting Adaptability of Polyacrylamide-based Amphoteric Cryogels Against Protein Molecules

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Abstract: Lysozyme, pepsin, ovalbumin, hemoglobin, and γ-globulin were chosen as templates to investigate the imprinting capability of amphoteric polyacrylamide cryogels. Prepolymerizing solutions contained acrylic acid and allyl amine, as well as acrylamide and N,N'-methylenebisacrylamide as functional monomers. As a result there were both acidic and basic functional groups in the polymers, facilitating effective interactions with likewise amphoteric proteins. The proteins differed greatly and cover wide scopes of molecular weights and isoelectric points. Regardless of the values of the molecular weights and isoelectric points, all the templates gave higher retentions on the molecularly imprinted polymer (MIP) tubes than on the non-imprinted polymer (NIP) tube. The MIP of lysozyme indicated the highest imprinting factor of 7.0, and that of γ-globulin showed the lowest, 2.0. The values of other proteins were intervenient. Conclusively, the amphoteric polyacrylamide cryogels were suitable imprinting materials for various proteins, and could potentially be useful for protein recognition, purification and depletion.

Key Words: Molecularly imprinted polymer; Amphoteric cryogel; Protein; Imprinting factor

1 Introduction

There are certain interactions between templates and the monomers used to synthesize molecularly imprinted polymer (MIP). During the polymerization procedures, these interactions are coordinated and accumulated, consequently resulting in cavities or sites to recognize the template molecules. By now the design, synthesis and applications of MIP have been widely studied[1–3].

As the translational products of biological genetic information, proteins exert many important functions in life processes. Protein imprint is always the key point of MIP research and is of great importance in molecular recognition, solid-phase extraction, disease diagnosis/therapy, proteomic research and environmental analysis[3–10]. To adapt various biological functions, the conformation and exterior shapes of proteins are subjected to a certain extent of alteration. In other words, the proteins are quite "flexible", hence rigid polymers are not the first choice of materials to imprint proteins. Additionally highly crosslinked polymers would make it difficult to remove the templates[11–14]. Protein imprint experiments were usually restricted to nano materials[15,16] or surface-imprinted polymers[17–20].

Cryogels are (super) macroporous materials made through cryogenic polymerization[21]. Puffy macroporous structures greatly reduce the mass transfer resistance inside the materials, which makes the imprint in cryogels similar to that of surface or nano particles, thus enhancing the mass transfer efficiency and facilitate the template wash-off[22–25]. Polyacrylamide-based materials possess good biocompatibility in favor of the treatments of biomacromolecules such as proteins, and are widely applied in life analysis. As well as generally used acrylamide and its derivatives, other monomers like vinyl alcohol and hydroxyethyl methylacrylate were used to prepare various MIPs.

If acrylic acid and allyl amine were introduced and copolymerized with acrylamide, polyacrylamide cryogels co-modified with both acidic and basic groups were obtained. These materials were amphoteric electrolytes just as protein

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molecules. In our previous study, a complex sample rather than standard proteins was used as "pending templates" to synthesize polyacrylamide-based MIPs. Specific recognizing sites against chicken egg white proteins were built in the polymers[26]. Through a homemade low pressure system, the MIPs were efficient to deplete parts of the abundant proteins including ovalbumin, lysozyme and ovotransferrin[27]. To better understand amphoteric polyacrylamide cryogels and provide valuable information for further research, we investigated the imprinting effects of amphoteric polyacrylamide cryogels for various proteins. Capillary chromatography columns were prepared based different protein-imprinted polymers to examine diverse retentions of the template molecules. Consequently, the synthesized MIPs exhibited good imprinting effects and specific recognizing abilities for the involved proteins regardless of their various molecular weights and isoelectric points.

2 Experimental

2.1 Instruments and reagents

Capillary electrochromatography was performed on a TriSep-2100 system (Unimicro Technologies, Inc., Shanghai, China). Scanning electron microscopy (SEM) was performed using an S-4800 microscope (Hitachi, Japan). Infrared (IR) spectrometry was performed on a Tensor27 spectrometer (Bruker, Germany). An FE20-K pH meter (Mettler Toledo, Shanghai, China) was used for acid-base titration.

Lysozyme (chicken egg white) was purchased from Biotopped Co. Ltd (Beijing, China), and ovalbumin from Sigma. γ-Globulin (bovine serum) was purchased from Solarbio Co. Ltd (Beijing, China). Pepsin (porcine gastric mucosa) was purchased from Sinopharm Chemical Reagents Co., Ltd (Shanghai, China). Bovine hemoglobin was purchased from Dinguo Biotechnology Co., Ltd (Shanghai, China). Acrylic acid, allyl amine, γ-methacryloxypropyl-trimethoxysilanesilane (γ-MAPS, 98%), acrylamide and *N*, *N*'-methylenebisacrylamide (BisAM) were purchased from Haopeng Chemical Plant (Jinan, Shandong, China). Sodium hydrogen sulfite (SHS), sodium dodecylsulfate (SDS) and ammonium peroxydisulfate (APS) were purchased from Sinopharm Chemical Reagents Co., Ltd (Shanghai, China). Capillaries (100 μm I.D., 360 μm O.D.) were purchased for Refine Chromatography Device Co., Ltd (Yongnian, Hebei, China). All the other reagents were of analytical grade.

2.2 Methods

2.2.1 Pretreatment of capillary

A capillary (about 3 m) was washed with 1.0 M H_2SO_4, deionized water, 1.0 M NaOH and methanol for 20 min respectively, and then dried at 120 ºC for 3 h. The cleaned capillary was filled with γ-MAPS (30%, *V*/*V* in methanol) and its ends were sealed with silica septa. This capillary was then put in a water bath of 80 ºC for 24 h. After that, the capillary was washed with methanol to remove the unreacted γ-MAPS and kept at 4 ºC for the further use.

2.2.2 Synthesis of polyacrylamide cryogels and preparation of capillary columns

Approximately 1.0 g of acrylamide, 0.5 g of BisAM, 250 μL of acrylic acid, 125 μL of allyl amine and 0.05 g of SHS were dissolved in 40 mL of phosphate buffer solution (10 mM, pH 7.4). The solutions were divided into 5 portions to imprint 5 templates (all at a concentration of 5 mg mL^{-1}). Ultrasonic degasification was carried out both before and after the addition of 0.03 g of APS in each of the solutions. γ-MAPS modified capillaries (12 cm) were filled with related solutions and put at a –20 °C refrigerator for 24 h. All the capillaries and cryogels were suffered from a water bath 60 °C for 2 h. The MIP columns were washed with a solution of 1 M NaCl (containing 10 g L^{-1} SDS) for 1 h. Non imprinted polymer (NIP) and the related column were prepared similarly, just without any protein templates.

2.2.3 Titration of polymers

Approximately 2 g of dried cryogel (MIP-2, pepsin as the template) was immersed in 10 mL of NaOH (1 M) for 5 min and cleaned with deionized water. Then the cryogel was put in 10 mL of deionized water and titrated with HCl (10 mM). To ensure a complete reaction between the protons and the cryogel, the titration should be performed slowly. After the addition of each batch of HCl (2 or 3 drops), the cryogel were stirred thoroughly for about 1 min. The pH values were recorded after the pH meter was stable.

2.2.4 Capillary electrochromatography

All the capillary columns were cut into 10-cm length, and connected to a bare capillary with a Teflon tube. The total length of the separation channel was 45 cm (25 cm from injection end to detection window). Proteins (5 mg mL^{-1}) were dissolved in a solution of 10 mM phosphate (pH 7.4). of Thiourea (0.2 mg mL^{-1}) was added as the electroosmotic flow marker. The running buffer was 10 mM phosphate (pH 8.0, containing 4 mM SDS). Injection was performed through 12 kV per 20 s. The voltage was 12 kV and the detection wavelength was 280 nm.

3 Results and discussion

Ideal protein imprinted polymers have biocompatibility and