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Improvement of recognition specificity of surface protein-imprinted magnetic microspheres by reducing nonspecific adsorption of competitors using 2-methacryloyloxyethyl phosphorylcholine

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ABSTRACT

A novel bovine serum albumin (BSA) surface-imprinted magnetic microsphere was fabricated by copolymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC), functional monomer acrylamide and cross-linking agent N,N′-methylenebisacrylamide on the surface of $Fe_3O_4@SiO_2$ microsphere. Here, MPC was creatively and strategically introduced to the protein-imprinted polymer as an assistant monomer to reduce nonspecific adsorption of competitive protein. 10 mol% of MPC was determined as the optimal content according to the imprinting factor. The structure and component of the obtained imprinted microsphere were studied by different characterization methods. The rebinding specificity experiments showed that the recognition specificity of the BSA-imprinted microsphere was greatly improved by introducing MPC. The corresponding adsorption capacity and imprinting factor were 21.79 mg/g and 8.32, respectively. More significantly, the selectivity coefficients of BSA to human serum albumin, ovalbumin, lysozyme, Cytochrome C and Ribonuclease A could reach up to 1.63, 5.23, 9.14, 7.43 and 7.23, respectively, benefited by the protein restricted access function of MPC polymer. Furthermore, this strategy had an excellent versatility and was also suitable for another template, lysozyme. The proposed strategy herein provided an effective means for improving recognition specificity of molecularly imprinted polymers and was expected to use in the improvement of detection sensitivity of molecularly imprinted biosensors.

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1. Introduction

Recently, with the development of the biomedical and pharmaceutical industries, the efficient separation and detection of target proteins from complex samples are of great significance [1,2]. To the best of our knowledge, they are still a challenge due to the complexity and conformational flexibility of protein [3]. Up to now, a series of strategies have been developed to overcome this challenge, such as chromatography, high-performance liquid chromatography, electrophoresis and microfluidic [4–7]. Although these methods have made progress, several limitations such as complex process, poor stability, high cost and inadequate coupling techniques, hinder the wide utilization of them [8–10]. Therefore, it is necessary to develop a simple and efficient technique for protein recognition and selective separation.

Molecular imprinting has proven to be a versatile approach [11–14]. It is used to prepare the artificial receptors with tailor-made recognition sites. These binding sites are complementary in shape, size and functionality to the target molecules. The resulting molecularly imprinted polymers (MIPs) possess a lot of advantages, such as low cost, easy preparation, excellent mechanical/chemical stability, high specificity and reusability [15,16]. In light of these characteristics, MIPs, similar to the artificial antibody, provide the possibility for the efficient and specific recognition of protein. In addition, as a promising material, it has attracted great interest, and been applied in a wide scope of applications, such as analytical separations, solid-phase extractions, chemical sensing and catalysis, drug delivery and artificial antibodies [10,17–19].

To date, many imprinting of small molecules have been reported and some of them are very excellent [20–23]. These MIPs exhibited high recognition ability to small target molecules as a man-made receptor. However, to our knowledge, the MIPs for proteins are not as satisfactory as the MIPs for small molecules. Large molecular size, flexible conformation and complexity of proteins make the imprinting process not only poor mass transfer but also less selectivity. Besides, the latter was also considered to have relation with

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non-specific adsorption of protein on the surface of material [8,10]. In response to these challenges, some versatile strategies have been developed, such as surface imprinting.

In surface imprinting, the imprinted binding sites are located at or near the surface of the MIPs, which facilitates the target molecules into and out of the imprinted sites [24]. In other words, the target proteins can be permitted to elute and rebind more effectively. Up to now, some protein-imprinted microspheres, prepared by surface imprinting, have been reported. For example, He's group [25] prepared lysozyme (Lyz)-imprinted microsphere simply via radical induced graft copolymerization on the surface of vinyl modified silica nanoparticles. Based on the above work, Lin's group [16] also synthesized Lyz surface-imprinted microspheres in the presence of acrylamide (AM) and N,N′-methylenebisacrylamide (MBA). Furthermore, they studied the effects of total monomer concentration, pH of buffer solution and buffer concentration on adsorption amount and imprinting factor. A novel bovine serum albumin (BSA) surface-imprinted magnetic microsphere [3], exhibiting good recognition capability to BSA, was prepared with Fe_3O_4 particle as the support using another synthesis method, atomic transfer radical polymerization. Although surface imprinting can overcome the mass transfer limitation, it cannot make the recognition specificity of protein-MIPs satisfactory for practical industrial application. Therefore, it is necessary to further improve the recognition specificity through the design of molecular chain structure in imprinted materials. Several approaches have been proposed to achieve this purpose. In Chen's work [26], the synthesis process was further studied toward enhancement of the imprinting performance by examining the effect of feed crosslinking degree and content of charged monomers. More promising, a novel strategy was developed by Liu's group [6], which combined the surface imprinting with assistant recognition polymer chains. Although these methods improved the recognition ability of protein-MIPs in a certain extent, the non-negligible non-specific adsorption of protein remains a big obstacle for getting an excellent selectivity. To the best of our knowledge, no studies have tried to introduce "protein resistant" to reduce the nonspecific adsorption of protein-imprinted material for improving the recognition ability.

2-Methacryloyloxyethyl phosphorylcholine (MPC), a methacrylate-functional monomer having a phospholipid polar group, inspired by the surface structure of cell membrane, is used to fabricate polymer biomaterials [27]. The resultant polymer possesses excellent biocompatibility, high lubricity and low friction. More significantly, it can reduce antibody denaturation and suppress protein adsorption effectively [28–31]. Owing to these properties, MPC polymers have potential application in various fields such as biosensors, drug carriers, soft contact lenses and vascular stents [32–35]. Herein, the inhibition of protein adsorption inspires our design about a novel BSA surface-imprinted magnetic microsphere and brings the motivation for further study. Our design is as follows. When MPC polymer is involved in protein-MIPs, the obtained imprinted microspheres can not only selectively recognize the template protein in complex matrix via imprinted sites but also reduce the competitive protein binding via MPC polymer. In this way, on the premise of guaranteeing the specific recognition of target protein, the selective recognition ability of protein-MIPs can be possibly improved by reducing competitive protein binding. Thus, in order to verify the feasibility of this strategy, we carried out the following work.

In this study, we had demonstrated the fabrication of a novel protein-imprinted magnetic microsphere by using BSA as template, AM as functional monomer and MBA as the cross-linker. Note that, different from previous studies, the highlight of this work was that the imprinted shell involved MPC polymer, which was promising to reduce the nonspecific adsorption of competitive proteins. The influence of MPC content on the adsorption capacity of the obtained microspheres was investigated in this work. Furthermore, the morphology and component of the obtained microspheres were characterized by transmission electron microscope (TEM), Fourier transform infrared spectrometry (FTIR), thermogravimetric analysis (TGA) and vibrating sample magnetometer (VSM). The adsorption capacity and selectivity of the obtained microspheres were discussed through adsorption isotherms and adsorption selectivity experiments. Then, the versatility of this method was demonstrated by using Lyz as template. Finally, the practicability for practical application in biology was further assessed by isolation and enrichment of the template protein from a standard protein mixture.

2. Experimental

2.1. Materials

2-Methacryloyloxyethyl phosphorylcholine (MPC) was obtained from J&K Chemical. Acrylamide (AM) and N,N′-methylenebisacrylamide (MBA) were purchased from Dingguo Biotech Ltd. Ammonium persulfate (APS), N,N,N,N-tetramethylenebis(acrylamide) (TEMED) and methacryloxy propyl trimethoxyl silane (MPTS) were provided by Sigma–Aldrich (Tokyo, Japan). Bovine serum albumin (BSA; Mw 66.4 kDa, pI 4.7), human serum albumin (HSA; Mw 66 kDa, pI 4.7), ovalbumin (OVA; Mw 46 kDa, pI 4.7) and Cytochrome C (Cyt C; Mw 13 kDa, pI 10.8) were obtained from Amresco (Solon, OH, USA). Ribonuclease A (RNase A; Mw 13.7 kDa, pI 7.8) and lysozyme (Lyz; Mw 13.4 kDa, pI 10.7) were the products of Sigma (St. Louis, MO).

2.2. Characterization

The morphologies and structures of the microspheres were characterized by transmission electron microscopy (TEM, Tecnai G2 20-S-TWIN). Samples were dispersed in ethanol at an appropriate concentration, cast onto a carbon coated copper grids and then dried under vacuum. Fourier transform infrared (FTIR) spectra were conducted at room temperature on a TENSOR27 FTIR spectrometer (Bruker). The samples were prepared by mixing the product with KBr and pressing into a compact slice. The inorganic content of the microspheres was obtained through thermogravimetric analysis (TGA-2950) under nitrogen atmosphere with a heating rate of $10\,^{\circ}C\,min^{-1}$ up to $720\,^{\circ}C$. The magnetic properties of the microspheres were assessed using a vibrating sample magnetometer (VSM, LakeShore 7307).

2.3. Preparation of BSA-imprinted magnetic microspheres

The BSA-imprinted magnetic microsphere (Fe_3O_4@SiO_2@BSA-MIPs) was prepared by the following steps (as shown in Fig. 1). The first step was the synthesis of the support, Fe_3O_4@SiO_2, which combined the advantages of magnetism and good biocompatible (Fig. 1A). The detailed steps referred to our previous work [36]. Then, the obtained Fe_3O_4@SiO_2 was modified by MPTS (Fig. 1B). In brief, 1 g Fe_3O_4@SiO_2 was suspended in a mixture of 75 mL ethanol and 50 mL deionized water, and the pH was adjusted to 9 by using ammonia solution (28 wt%). Subsequently, 0.3 g MPTS was added and reacted with Fe_3O_4@SiO_2 for 24 h at room temperature to introduce double bond functional groups. The resultant MPTS-modified magnetic microspheres were purified by ethanol and deionized water and freeze-dried for subsequent use. In the next step, using BSA as the target molecule, the imprinted shell was coated on the surface of MPTS-modified Fe_3O_4@SiO_2 through grafting copolymerization in the presence of AM, MPC and MBA (Fig. 1C). The details were as follows: 0.3 g MPTS-modified Fe_3O_4@SiO_2 was dispersed in 20 mL phosphate buffer (0.02 mol/L, pH 7.0)

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