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Effect of crosslinking degree and thickness of thermosensitive imprinted layers on recognition and elution efficiency of protein imprinted magnetic microspheres

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ABSTRACT

Crosslinking degree is difficult to determine on the premise of simultaneously considering response performance and recognition capability in the thermosensitive imprinted materials due to their mutually contradictory relationship. In addition, the effect of thickness of thermosensitive imprinted layers on the recognition of target protein is also difficult to evaluate quantitatively. To solve these two problems, a series of thermosensitive magnetic imprinted microspheres with different crosslinking degree and imprinted layer thickness were fabricated. The adsorption results showed that imprinted layer thickness of 17 nm was the most appropriate for BSA imprinting. Crosslinking degree of 20% was determined as the balance point by simultaneously considering response performance and recognition capability. Under such conditions, the elution efficiency after once washing process was up to 78.60%, which guaranteed the satisfactory regeneration capability. The adsorption capacity and the imprinting factor could reach 42.01 mg/g and 3.41, respectively, which were greatly increased compared to those imprinted materials reported previously. Furthermore, the practical separation performance of the imprinted microspheres was investigated by separating BSA from the protein mixture sample, and the results were exciting. All these results indicated that these imprinted microspheres were expected to be applied to the rapid isolation and purification of BSA.

1. Introduction

Recently, proteomics is one of the most important areas of research. It has a very close relationship with human health. With the in-depth study of proteomics, it is found that the separation and identification of proteins play an important role in the development of proteomics. At present, the selective recognition of proteins is almost entirely based on the antigen–antibody reactions. Although the recognition capability of this method is considerable, it is usually very complex and involves different techniques with many time-consuming steps [1]. Furthermore, the high cost and difficult acquisition of antibodies also somewhat hindered the promotion of the technology [2]. Therefore, it is highly desirable to develop a universal, inexpensive and facile method, to achieve the fast and high selective separation of the target proteins.

Molecular imprinting is an emerging separation technology. It has the following three characteristics [3–5]: (1) universality—corresponding molecular imprinted polymers (MIPs) can be prepared depending on the type of template molecule to meet the separation requirements of various targets; (2) recognition—MIPs possess the tailor-made recognition sites which are complementary in shape, size and functionality to the target molecules; (3) practicability—comparing with enzymes, antibodies, and natural receptors, MIPs exhibit easier acquisition, higher stability, longer life and lower cost. Due to these three unique advantages, molecular imprinting has been applied in various fields, such as analytical separations, solid-phase extractions, chemical sensing and catalysis, drug delivery and artificial antibodies [6–9]. In recent decades, the imprinting of small molecules has been well achieved. However, protein imprinting still presents challenges, due to the large molecular size, flexible conformation, complex structure, solubility and sensitivity to the environment

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of proteins. In response to these challenges, although many versatile strategies have been developed [10–17], new and easier approaches for protein imprinting are still highly demanded.

Thermo-responsive polymer is an intelligent polymer. It is sensitive to temperature. Among a variety of thermo-responsive polymers, poly(N-isopropylacrylamide) (PNIPAM) is one of the most widely investigated polymer. The hydrophobic/hydrophilic character of its molecular chain can change reversibly with temperature. As a result, this thermo-responsive polymer can undergo a reversible swelling-shrinking cycle in response to external temperature. Based on the unique volume phase transition property, when thermo-responsive polymer is integrated with MIPs, the resulting imprinted polymer is able to control the uptake and release of template molecules in response to external temperature. Such a strategy could overcome the problems of slow mass transfer and incomplete release in traditional MIPs [18,19], especially protein MIPs.

At present, some thermo-responsive protein imprinted polymers have been reported in previous works. For example, Pan et al. [20] fabricated a protein-imprinted spherical nanogel particle with excellent specificity via aqueous precipitation polymerization with the aid of sodium dodecyl sulfate by using lysozyme (Lyz) as the template molecule and NIPAM as the major monomer. The rebinding and release characteristics of the Lyz-imprinted nanogels showed dramatic temperature-dependence, with clear on–off transition around the lower critical solution temperature (LCST) of PNIPAM. Using the similar strategy, Zhang et al. [21] prepared a novel thermo-responsive molecularly imprinted hydrogel composed of NIPAM and acrylamide by using BSA as the template protein. Unlike the above study, an own-made modified water-soluble N-maley chitosan was chosen as the cross-linker in the work to improve the recognition capability. Similarly, for getting higher recognition capability, a metal chelate monomer, N-(4-vinyl)-benzyl iminodiacetic acid, forming coordination complex with the template protein in the presence of Cu^{2+}, was introduced in Qin's work [22]. The obtained macroporous thermo-responsive hydrogel exhibited excellent specific recognition ability to the target protein. This kind of approach was suitable for the certain protein with surface histidines. Although these imprinted materials showed satisfactory results in the identification and controllable release of the target protein, they still had some shortcomings, such as poor mechanical strength and single function.

For getting higher mechanical strength, simultaneously giving the imprinted materials more functional characteristics, preparing the thermo-responsive imprinted shell on the surface of functional inorganic particles is a good strategy. For instance, Qin's group [23] prepared PNIPAM-coated molecularly imprinted beads by two-step surface-initiated living-radical polymerization using mesoporous chloromethylated polystyrene beads as the support for controlling the release of protein. Moreover, another thermo-responsive protein-affinity material was also developed by them using CdTe quantum dots (QDs) as the support [24]. The obtained imprinted materials combined the merits of molecular imprinting technology, the thermo-sensitive characteristic of the NIPAM and the fluorescent property of the QDs. This combination was more conducive to the detection and sensing of target protein. In addition, a novel BSA surface-imprinted thermo-responsive magnetic composite microsphere was prepared in our previous work [25]. The obtained magnetic composite imprinted microspheres could not only selectively recognize the template molecules in response to external temperature but also could be easily separated by an external magnetic field.

In these research works [20,22–25], the influences of temperature on the rebinding capacity and releasing ability were investigated and the corresponding laws were provided. However, as we all know, crosslinking degree is an important factor for the temperature sensitivity of thermo-responsive polymer. When crosslinking degree increases, the temperature sensitivity will be greatly reduced [26,27]. Thus, a high crosslinking degree is not conducive to the release of template protein, which will reduce the regeneration performance of imprinted sites. Note that, it does not mean that lower crosslinking degree is better. The reason, mentioned by the previous reports [21,28,29], is that the increase of crosslinking degree in low range is beneficial to the improvement of recognition ability. This conclusion caught our attention and the same phenomenon was also observed in our previous work by adjusting the original formula of BSA surface-imprinted thermo-responsive magnetic composite microsphere. According to the above description, when crosslinking degree is in low range, temperature sensitivity and recognition ability are mutually contradictory. So, with this in mind, it is a challenge to determine the crosslinking degree in the thermosensitive imprinted materials. In addition, there is another challenge, that is, the effect of thickness of thermosensitive imprinted layers on the recognition of target protein is difficult to evaluate quantitatively. To overcome these two challenges, our present research work, to the best of our knowledge, is the first one to be carried out.

In this work, a series of thermosensitive BSA surface-imprinted magnetic microspheres were fabricated by the surface grafting copolymerization method. The imprinted layer was composed of NIPAM, methacrylic acid (MAA) and N,N′-methylenebisacrylamide (MBA). NIPAM was chosen as the temperature-sensitive component, MAA was the functional monomer, and MBA was the cross-linker. The most appropriate imprinted layer thickness for BSA was investigated, and the balance point of crosslinking degree was determined by simultaneously considering response performance and recognition capability. The thermosensitivity of imprinted layer was reflected by the elution efficiency. Then, the resultant surface-imprinted magnetic microspheres were evaluated by investigating the adsorption kinetics, the adsorption isotherms and the specificity. At last, the practical performance for biological application was further assessed by separating BSA from the protein mixture sample.

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (TEOS), methacryloxy propyl trimethoxyl silane (MPTS), ammonium persulfate (APS) and N,N,N,N-tetramethylenebis(acrylamide) (TEMED) were provided by Sigma–Aldrich (Tokyo, Japan). N-Isopropyl acrylamide (NIPAM) was purchased from Acros Organics (Morris Plains, NJ, USA). Methacrylic acid (MAA) and N,N-methylenebisacrylamide (MBA) were supplied by Dingguo Biotech Ltd (Beijing, China). Bovine serum albumin (BSA), Human serum albumin (HSA), Ovalbumin (OVA), Cytochrome C (Cyt C), Ribonuclease A (RNase A) and Lysozyme (Lyz) were obtained from Amresco (Solon, OH, USA). Other chemicals were analytically pure and used as received.

2.2. Characterization

The morphologies and structures of the microspheres were determined by transmission electron microscopy (TEM, JEOL JEM-3010). 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 recorded with a TENSOR27 FTIR spectrometer (Bruker) in the range of 4000–400 cm^{-1} by use of KBr pellets. The polymer contents of the microspheres were obtained through thermogravimetric analysis (TGA, Q50, TA instruments) under nitrogen atmosphere