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Preparation and characterization of bovine serum albumin surface-imprinted thermosensitive magnetic polymer microsphere and its application for protein recognition

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ABSTRACT

A novel bovine serum albumin surface-imprinted thermosensitive magnetic composite microsphere was successfully prepared by the surface grafting copolymerization method in the presence of temperature-sensitive monomer N-isopropylacrylamide (NIPAM), functional monomer methacrylic acid (MAA) and cross-linking agent N,N′-methylenebisacrylamide (MBA). The structure and component of the thermo-sensitive magnetic molecularly imprinted microsphere were investigated by transmission electron microscopy (TEM), Fourier transform infrared (FT-IR), vibrating sample magnetometer (VSM) and thermogravimetric analysis (TGA). The results of thermosensitivity, adsorption capacity, selectivity and reusability showed the formation of a thermosensitivity grafting polymer layer P(NIPAM–MAA–MBA) on the surface of Fe_3O_4@SiO_2 and the good adsorption capacity and specific recognition for template protein. When the adsorption temperature was higher than the lower critical solution temperature (LCST) of poly (N-isopropylacrylamide) (PNIPAM), shape memory effect of imprinted cavities would be more effective. In other words, it was more conducive to capture template molecules under this condition and the imprinting factor would be higher. On the other hand, when the desorption temperature was lower than LCST of PNIPAM, the decrease of shape memory effect between imprinted cavities and template molecules would facilitate the release of template molecules from the imprinted cavities. Based on this property, the adsorption and desorption of template molecules could be regulated by system temperature indirectly which benefited from the existence of thermosensitivity imprinting layer.

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1. Introduction

Molecular imprinting is an emerging technique which creates sites that are complementary in shape, size and functionality with respect to the template molecules in recent years (Wulff et al., 1997; Lin et al., 2012; Kyoko et al., 2013). The excellent mechanical/chemical stability, low cost, easy preparation and high specificity of molecularly imprinted polymers (MIPs) prepared by molecular imprinting render them applied to chromatographic separation, solid-phase extraction, drug delivery, medical diagnostics and biosensors (Tan et al., 2008; Kyoko et al., 2013; Ali et al., 2013). Most of the published reports were concerned with small template molecules, and some of them were very excellent. However, there were few reports about protein imprinting, especially large protein imprinting. The major challenges contain the following: (1) huge molecular size of protein renders it difficult to transfer; (2) sensitivity of biomacromolecule and complex structure of protein make the imprinting process less selective. To resolve these problems, some methods have been developed, such as surface imprinting (Zhang et al., 2010; Hua et al., 2009; He et al., 2010; Zhou et al., 2010) and epitope imprinting (Nishino et al., 2006; Tai et al., 2005).

Magnetic microspheres have the merits of high dispersion stability, excellent biocompatibility and high magnetic susceptibility. In addition, they can be easily modified with different functional groups according to research need. So they have received more and more attention in various application fields such as catalysis, clinic diagnosis and therapy, bioseparation and targeted drug delivery in recent years (Ma et al., 2012a; Gupta and Gupta, 2005; Gu et al., 2006; Gao et al., 2009). When magnetic microspheres are coated with MIPs, the obtained magnetic composite imprinted microspheres can not only selectively recognize the template molecules in complex matrix through imprinted polymer shell but also can be easily separated by an external magnetic field through magnetic nucleus (Tan et al., 2008; Lu et al., 2006).

Temperature-sensitivity polymer which is sensitive to temperature belongs to intelligent polymer. Among them, poly(N-isopropylacrylamide) (PNIPAM) is one of the most widely investigated polymers. It is well known that Lower Critical Solution

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Temperature (LCST) of PNIPAM is 32 °C. Consequently, when the temperature increases to 32 °C, it switches from a hydrophilic, coil state to a hydrophobic, collapsed state. Moreover, this transformation between hydrophilic and hydrophobic is reversible. When temperature-sensitivity is integrated with MIPs, the ability of resulting imprinted polymer in capturing and releasing template molecules can be adjusted by external temperature.

Up to now, some magnetic or temperature-sensitive molecularly imprinted microspheres have been reported in previous works. For example, Jing et al. (2010) synthesized lysozyme surface-imprinted magnetic microspheres in the presence of acrylamide (AM), methyl acrylic acid (MAA) and N,N'-methylenebisacrylamide (MBA) based on surface grafting copolymerization method. Gao et al. (2011) selected Lyz, BHb, BSA and RNase A as template molecules and fixed them onto the aldehyde-modified Fe_3O_4@SiO_2 through imine bond formation between the aldehyde groups and amine groups on the proteins. Subsequently, siloxane co-polymerization on the Fe_3O_4@SiO_2–protein complex surface from 3-aminopropyl triethoxy silane (APTES) and octyltrimethoxysilane resulted in a polymeric network model around the template proteins. Then template protein surface-imprinted magnetic microspheres were obtained. It turned out that adsorption capacity mainly depended on the hydrophobic interaction and the charge effect. Ran et al. (2012) prepared a novel temperature-sensitive BSA molecular imprinted hydrogel composed of 2-acrylamido-2-methyl-propanosulfonic acid (AMPS), NIPAM, AM and MBA under two different temperatures (25 °C and −20 °C) by free-radical cross-linking copolymerization in aqueous solution. The results showed that the shape memory and the charge effect were the major factors for the recognition. However, there were few reports with respect to the combination of all three elements (magnetism, temperature-sensitivity and molecularly imprinting). Gai et al. (2011) synthesized BSA surface-imprinted magnetic polymer exhibiting higher adsorption capacity and selectivity to BSA through the atomic transfer radical polymerization (ATRP) method in the presence of common monomer NIPAM, functional monomer N-[3-(dimethylamino)propyl]-methacrylamide (DMAPMA) and MAA. Nevertheless, temperature-sensitivity of imprinted polymer was not discussed. Furthermore, the process of preparation by ATRP was cumbersome and strict.

In this work, bovine serum albumin surface-imprinted polymer microspheres with both thermosensitive and magnetic have been prepared by the surface grafting copolymerization method. The shell of imprinting was composed of NIPAM, MAA and MBA. Here, NIPAM was chosen as the temperature-sensitive component which allowed for swelling and shrinking with response to temperature changes, MAA was the functional monomer on account of the affinity of carboxyl groups toward amide groups of BSA, and MBA was the cross-linking agent. The morphology and composition of surface-imprinted thermosensitive magnetic polymer microspheres were investigated by transmission electron microscope (TEM), Fourier transform infrared spectrometry (FTIR), vibrating sample magnetometer (VSM) and thermogravimetric analysis (TGA). Thermosensitivity of the BSA-imprinted magnetic microspheres was investigated in this work. Furthermore, the adsorption capacity and selectivity of Fe_3O_4@SiO_2@BSA-MIP (NIP) were discussed through adsorption kinetics, adsorption isotherms, reusability tests and adsorption selectivity experiments.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS) and γ-methacryloxypropyltrimethoxysilane (KH-570) were obtained from Sigma (St. Louis, MO, USA). NIPAM was supplied by Acros Organics (Morris Plains, NJ, USA). N,N-methylenebisacrylamide (MBA), methacrylic acid (MAA), ammonium persulfate (APS) and N,N,N,N-tetramethylenebis(acrylamide) (TEMED) were provided by Sigma-Aldrich (Tokyo, Japan). Bovine serum albumin (BSA), human serum albumin (HSA), ovalbumin (OVA), cytochrome C (Cyt c), ribonuclease A (RNase A) and lysozyme (Lyz) were purchased from Amresco (Solon, OH, USA).

2.2. Characterization

Fourier Transform Infrared (FTIR) spectra were acquired on a TENSOR27 FTIR spectrometer (Bruker). The samples were prepared by mixing the products with KBr and pressing into a compact pellet. Morphology and structure of the microspheres were observed in Transmission Electron Microscope (TEM, JEOL JEM-3010). The magnetic properties of magnetic microspheres were assessed with a vibrating sample magnetometer (VSM, LakeShore 7307).The polymer content of Fe_3O_4@SiO_2@BSA-MIP(NIP) was determined through thermogravimetric analysis (TGA, Q50, TA instruments) in the temperature range from room temperature to 900 °C with a heating rate of 10 °C/min under nitrogen atmosphere.

2.3. Preparation of Fe_3O_4@SiO_2@BSA-MIP(NIP)

Fig. 1 illustrates the synthesis schematic diagram of Fe_3O_4@SiO_2@BSA-MIP. The Fe_3O_4 microspheres were prepared through the solvothermal method according to the literature (Ma et al., 2012b). The resulting microspheres were coated with a thin SiO_2 film (Fe_3O_4@SiO_2) through the Stöber process (Luo et al., 2010). Briefly, 1 g Fe_3O_4 was dispersed in a mixture of 50 mL ethanol, 5 mL deionized water and 6 mL of 28 wt% concentrated ammonia aqueous solution to form a suspension liquid. Subsequently, 3 g TEOS was added to the suspension liquid in drops. After stirring at room temperature for 12 h, the obtained products were freeze-dried after washing by ethanol and deionized water. Thus Fe_3O_4@SiO_2 microspheres were obtained.

Then, 1 g Fe_3O_4@SiO_2 was dispersed in a mixture of 75 mL ethanol and 25 mL deionized water. The pH of mixture was adjusted to 4 using acetic acid, whereafter 25 mL of 1.2 wt% concentrated KH-570 ethanol solution was added to the mixture under the protection of nitrogen. The solution was stirred for 24 h at room temperature. The products Fe_3O_4@SiO_2-C═C were collected by an external magnetic field, and freeze-dried for subsequent using after washing by ethanol and deionized water.

Fe_3O_4@SiO_2@BSA-MIP was synthesized by precipitation polymerization. First, 84.8 mg NIPAM, 21.5 mg MAA, 7.7 mg MBA, and 20 mg BSA were dissolved in 20 mL phosphate buffer (0.2 mol/L, pH=7.0). After that, 0.3 g Fe_3O_4@SiO_2-C═C was dispersed in the above solution. When the temperature rises to 35 °C, 10 mL phosphate buffer (0.2 mol/L, pH=7.0) containing 10 mg APS and 10 μL TEMED was added for polymerization over 24 h.

The preparation method of Fe_3O_4@SiO_2@BSA-NIP was the same as Fe_3O_4@SiO_2@BSA-MIP, but the former was prepared with no BSA as template molecule.

2.4. Elution of template protein

The obtained Fe_3O_4@SiO_2@BSA-MIP microspheres were rinsed several times with purified water and separated by an external magnetic field to remove the unreacted monomer and the entrapped template molecules, whereafter, they were washed repeatedly with the solution containing SDS (10%, w/v) and acetic acid(10%, v/v) to remove the embedded BSA template molecules at room temperature. The complete removal of BSA from the Fe_3O_4@SiO_2@BSA-MIP was confirmed by a UV-2550 (Shimadzu) spectrophotometer at 276 nm detection wavelength.

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