Preparation and Recognition Properties of Bovine Hemoglobin Magnetic Molecularly Imprinted Polymers

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Received: October 21, 2009; Revised Manuscript Received: February 4, 2010

A simple method for the preparation of core−shell micro/nanostructured magnetic molecularly imprinted polymers (MIPs) for protein recognition is described. Magnetic MIPs were synthesized by copolymering γ-aminopropyltrimethoxysilane and tetraethyl orthosilicate at the surface of Fe_3O_4 nanospheres, which were directly covalently bound with template molecule, bovine hemoglobin (BHb), through imine bond. Transmission electron microscopy and scanning electron microscopy images showed that the Fe_3O_4 nanospheres with diameter about 50−150 nm were coated with the MIPs layer with average thickness about 10 nm, which enabled the magnetic MIPs to have a sensitive and fast magnetic response. The proximity between the thickness of MIPs layer and the spatial size of BHb indicated that the imprinted sites almost situated at the surface of magnetic MIPs, leading a rapid adsorption saturation within 1 h. And the adsorption amounts of magnetic MIPs toward BHb were estimated to be 10.52 mg/g at pH 6.5, which was 4.6 times higher than that of magnetic nonmolecularly imprinted polymers. Meanwhile, the result of selective test showed that the magnetic MIPs had an excellent recognition capacity to BHb compared to the other nontemplate proteins. Except for the spatial size complementary between BHb and the binding sites in magnetic MIPs, the electrostatic interaction also was proven to be an important factor for recognizing the imprinting molecule.

Introduction

Molecular imprinting, first constructed by Wulff[1] and Mosbach,[2] is a well established and facile technique for synthesizing molecularly imprinted polymers (MIPs) with specific molecular recognition capacity. Owing to the complementarity in shape and binding sites, the created nanocavities can act as artificial antibodies and exhibit high selectivity toward the imprinted molecules, including a large and diverse set of important organic molecules,[3,4] bioorganic molecules,[5,6] or metal ions.[7,8] Advantages such as chemical, mechanical, and thermal stability together with high selectivity for the template molecules enable MIPs to apply in wide fields, such as chiral separation, solid phase extraction, biosensors, and drug controlled release.[9–15]

Although MIPs have been successfully developed against a wide range of small molecules, the imprinting of macromolecules like proteins has proven to be more problematic and development was considerably slow.[16] The problems associated with imprinting of relatively unstable three-dimensional conformations, possible rearrangement processes, and poor solubility of the protein molecule in organic solvents are usually the main reasons why the imprinting of proteins remains difficult.[17] In spite of these difficulties, there is still a strong attempt to prepare protein imprinted polymers for the use in bioenrichment, bioseparation, biosensors, and so on.[18–20] A thermosensitive macroporous hydrogel showing selectivity for lysozyme was developed based on metal coordination interaction by Zhang et al.[21] The interaction of the imprinted hydrogel to the template protein could be switched between the coordinate effect and the electrostatic effect by adding or not adding Cu ions. Zhao and co-workers reported a novel stimuli-responsive protein imprinted polymer for selective recognition of bovine serum albumin by using *N*-isopropylacrylamide and *N*-[3-(dimethylamino)propyl]-methacrylamide as functional monomers.[22] Different approaches have been developed to synthesize protein imprinted polymers, the most effective of which should be surface imprinting technique.

Surface imprinted technique can not only overcome above disadvantages, but also resolve the problems raised from the bulk MIPs prepared by traditional method, such as time and labor requirement, long response time, and poor site accessibility for the template molecule.[23–25] Because of the imprinting sites situated at the polymer surface or close to the surface, the prepared MIPs have the properties such as template removal, good accessibility to the target species, and low mass-transfer resistance.[26–29] Surface-imprinted conducting polymer microrods for avidin recognition has been synthesized by Gyurcsányi et al.[30] Haupt described the generation of surface-bound nanofilaments with a high aspect ratio by nanomolding on a nanoporous template surface.[31] The imprinted polymers surface exhibited specific binding for template myoglobin as revealed by fluorescence microscopy.

Fe_3O_4 particles, as special biomolecule immobilizing carriers, have gained wide attraction. Because of their magnetically susceptible properties, functional materials embedded with iron oxide particles have been demonstrated for enrichment, biological separation, and capture of rare cells, proteins, and nucleic

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10.1021/jp910060c
Published on Web 02/25/2010

acids. When Fe_3O_4 particles are encapsulated inside of MIPs, the resulting polymer material will have magnetically susceptible characteristics, and can be easily separated by external magnetic fields after they had finished their adsorption and recognition. The successful applications of magnetic MIPs in the recognition of biomolecules have also been reported. Tong and co-workers reported the preparation of core−shell magnetic MIPs prepared by miniemulsion polymerization for ribonuclease A recognition.[32] Yang and co-workers synthesized magnetic MIPs in the nanoporous of alumina membrane for theophylline recognition.[33]

Herein, the surface imprinting and sol−gel technique were combined to prepare the magnetic MIPs for protein recognition. To simplify the synthesis process, the Fe_3O_4 nanospheres were directly functionalized with amine groups instead of being coated with inorganic polymers.[34] Model protein, bovine hemoglobin (BHb) was directly covalently grafted on the modified Fe_3O_4 nanospheres surface with amine groups by glutaraldehyde. And then the polymerization reaction took place on the nanosphere surface. The synthesis magnetic MIPs were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), ransmission electron microscopy (TEM), scanning electron microscopy (SEM), and vibrating sample magnetometer (VSM). The magnetic responsivity of magnetic MIPs was strong enough to purify and separate magnetic MIPs by the external magnetic field. And the adsorption kinetics, special adsorption, and selective recognition capacities of magnetic MIPs were also investigated. Easy preparation, chemical stability, recognition capacity of template molecule, as well as to discriminate molecules with variations in charge of the synthesized magnetic MIPs, make this approach attractive and broadly applicable in bioenrichment, separation, and sensors.

Experiment and Methods

Chemicals. Bovine hemoglobin (BHb), bovine serum albumin (BSA), lysozyme (Lyz), ribonuclease A (Rnase A), and albumin egg (EA) were purchased from Sigma. γ-aminopropyltriethoxysilane (γ-APS) was purchased from Jintan Eastchina Coupling Agent Factory, China. All other reagents used were of AR grade and used as received without further purification. Doubly distilled water was used in this work.

Instruments. The morphology of magnetic MIPs was observed by TEM (JEOL IEM-200CX) and SEM (S4800). All FTIR measurements were performed on a BRUKER IFS 66/S Fourier Transform spectrometer. Ultraviolet visible (UV−vis) absorption spectra of proteins were recorded by a UV-2401PC spectrometer. The crystal structure of magnetic MIPs was characterized by XRD (Philips) using Cu Kα radiation. Magnetic measurements were carried out using a VSM (7300, Lakeshore) under a magnetic field up to 10 kOe. ζ-potential of the polymers were determined on a Zetasizer Nano Z (Malvern Instruments Ltd.) in phosphate buffer solution (PBS, pH 6.5−7.4, 0.1 mol/L). Energy dispersive X-ray spectroscopy (EDX) (SAIMADZU, SSX-550 instrument) was used to observe the chemical composition of products.

Preparation of Fe_3O_4 Nanospheres Covalent Bonding with BHb. $FeCl_3 \cdot 6H_2O$ (1.35 g, 5 mmol) was dissolved in ethylene glycol (40 mL) to form a clear solution, followed by the addition of NaAc (3.6 g) and polyethylene glycol (1.0 g). The mixture was stirred vigorously for 30 min and then sealed in a teflon-lined stainless-steel autoclave (50 mL capacity). The autoclave was heated to maintain a temperature at 200 °C for 8 h, and then allowed to cool to room temperature. The black products were washed several times with ethanol and dried at 60 °C for 6 h.[35]

Amino group functionalization of Fe_3O_4 nanospheres was achieved by a direct silanizing of Fe_3O_4 nanospheres with γ-APS.[36] The Fe_3O_4 nanospheres were dispersed into 100 mL of toluene, and the mixture solution of 2 mL of γ-APS and 2 mL of triethylamine was dropwise added to the suspension within 10 min. The ferrofluid suspension was refluxed under mechanical stirring for 12 h with N_2 gas. The modified nanospheres were magnetically collected and redispersed into ethanol by ultrasonication. After being washed with ethanol for several times, the nanoshperes were dried in vacuum, obtaining amino group functionalized Fe_3O_4 ($Fe_3O_4-NH_2$).

After 0.5 g of $Fe_3O_4-NH_2$ was dispersed in 50 mL of PBS (pH 7.0, 0.1 mol/L) under ultrasonication, 2 mL of 25% glutaraldehyde was dropwise added into the solution. Then the mixture solution was oscillated with 200 rpm/min for 12 h at 25 °C. The obtained product was washed with doubly distilled water to give Fe_3O_4-CHO. Then 50 mg of BHb was added

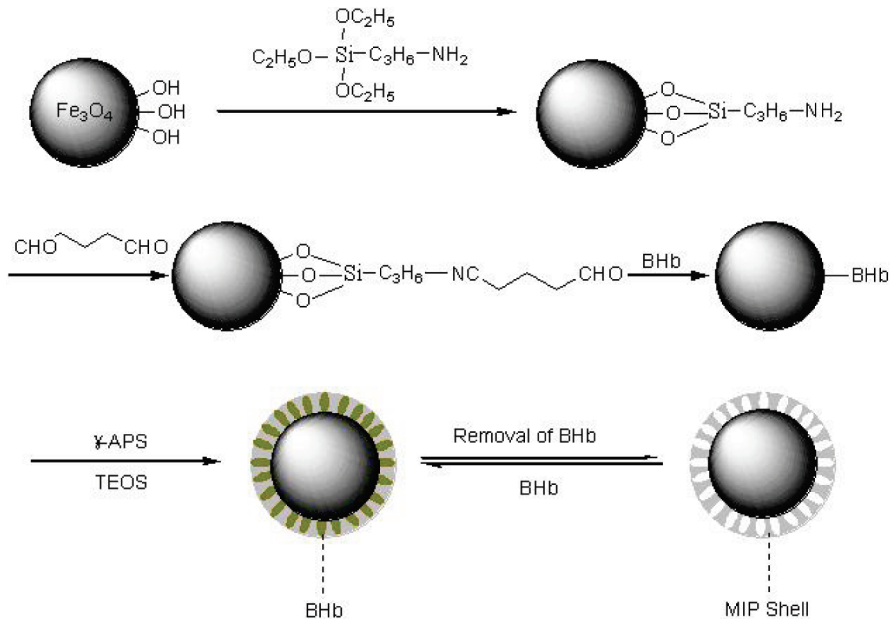

Figure 1. Schematic of preparation of magnetic molecular imprinted polymers for BHb recognition.

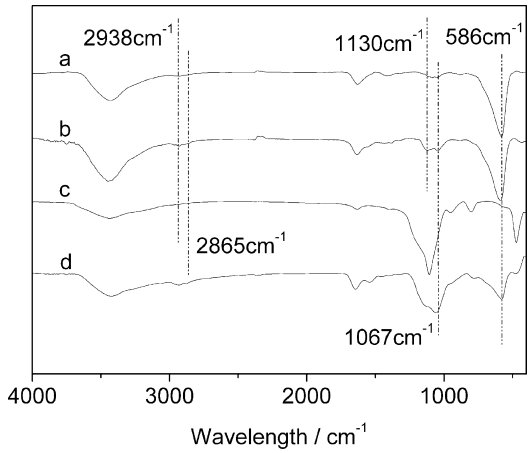

Figure 2. FTIR spectra of the Fe_3O_4 nanospheres (a), $Fe_3O_4-NH_2$ (b), pure MIPs (c), and Magnetic MIPs (d).

into 100 mL of PBS (pH 7.0, 0.1 mol/L) containing the dispersed Fe_3O_4-CHO. The mixture was incubated in an oscillator with ice bath for 12 h. Product was separated by external magnet and washed with PBS, labeling as Fe_3O_4-BHb.

Preparation of Magnetic MIPs. The mixture of 1 mL of tetraethoxysilane (TEOS) and 1 mL of γ-APS (TEOS/γ-APS = 1:1 in molar ratio) was dropped slowly into 100 mL of Tris (pH 8.5, 0.1 mol/L) buffer solution containing 0.5 g Fe_3O_4-BHb with vigorously mechanical stirring. The temperature was kept at 25 °C for 12 h under N_2 gas. The resulting polymers were collected by external magnet and were washed with distilled water until the supernate reached neutral. The resulting supernate was replaced with the mixture solution of 50 mL of water and 160 mL of MeOH and then 0.254 g of sodium bicarbonate was added. The suspension was mechanically stirred at 25 °C for 20 h under N_2 gas to extract the template protein.[37] The obtained product was repeatedly washed with distilled water and dried in the vacuum desiccator for 24 h before used.

For comparison, magnetic nonmolecularly imprinted polymers (NIPs) were prepared by the same procedure, only using Fe_3O_4-CHO to replace Fe_3O_4-BHb in the polymerization process.

Binding Experiments. Twenty milligrams magnetic MIPs was added into 5 mL tube and was suspended in 2.0 mL of PBS with specific initial concentrations of BHb ranging from 0.05 to 1.0 mmol/L. After shaken at 25 °C for 2 h, the mixture was separated by external magnet. The concentration of free BHb in the supernate was measured by UV−vis at 405 nm. The amount of BHb bound to magnetic MIPs was calculated by subtracting the amount of free BHb in the supernate from the amount of BHb initially added. The adsorption kinetics of BHb toward magnetic MIPs was investigated by changing the adsorption time from 0 to 150 min and the initial concentration of BHb was kept constantly at 0.4 mg/mL. The selectivity of the magnetic MIPs was investigated by using EA, Lyz, Rnase A, and BSA as the comparative proteins with initial concentration of 0.4. mg/mL. The concentration of comparative proteins in supernate was measured by UV−vis at 280 nm.

Results and Discussion

Characterization of Magnetic MIPs. With magnetically susceptible characteristics, high-mechanical intensity, and large surface area, Fe_3O_4 nanosphere is an ideal matrix material for the preparation of MIPs by using surface imprinting technique. The amine groups, aldehyde groups, and BHb were grafted on

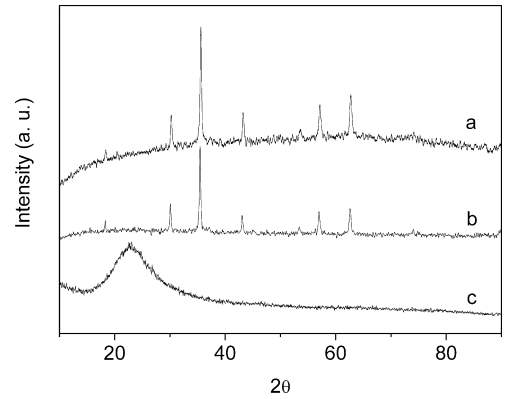

Figure 3. X-ray diffraction patterns of Fe_3O_4 nanospheres (a), magnetic MIPs (b), and MIPs (c).

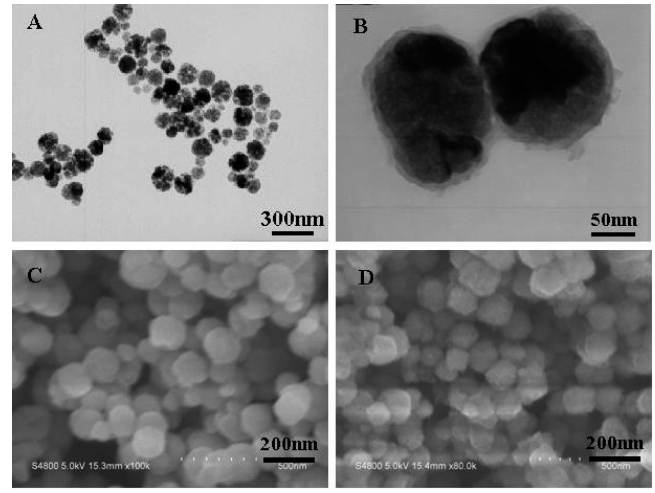

Figure 4. TEM images of Fe_3O_4 nanospheres (A) and magnetic MIPs (B), and SEM images of Fe_3O_4 nanospheres (C), and magnetic MIPs (D).

the Fe_3O_4 surface successively by the former three step synthetic procedures as shown in Figure 1. Chemical modification of inorganic materials using alkoxysilanes was reported.[38–40] The surface hydroxyl groups of Fe_3O_4 nanospheres were first reacted with the ethoxy groups of aminoproxyl triethoxysilane to form amino groups functionalized Fe_3O_4, which subsequently reacted with glutaraldehyde, to yield an aldehyde groups modified Fe_3O_4. The aldehyde groups were then reacted with amino groups in protein by covalent bond to graft the protein on Fe_3O_4 surface. The fourth step in Figure 1 was the magnetic MIPs synthetic process through a simple co-condensation approach of TEOS and γ-APS where γ-APS and TEOS were used as functional monomer and cross-linker, respectively.

FTIR spectroscopy was used to study the chemical structure of the functionalized Fe_3O_4 nanospheres. A sharp and strong Fe−O stretching peak (~586 cm^{-1}) was observed for all surface-modified samples, indicating that the main structure was not changed by the modification,[41] as shown in Figure 2. The absorption bands at ~2865 cm^{-1} and 2938 cm^{-1} (C−H stretching) of $Fe_3O_4-NH_2$ indicated the presence of $-CH_2$ groups[36] and the successful modification of γ-APS. As a result, the amino group was introduced onto the Fe_3O_4 surface. In curve d, the presence of Fe_3O_4 core could be observed by the strong absorption band of Fe−O bond of naked Fe_3O_4 at around 586 cm^{-1}. Meanwhile, the existence of MIPs shell could be confirmed by the appearance of characteristic adsorption bands of stretching vibration of SiO-H and Si−O−Si of silica matrix.

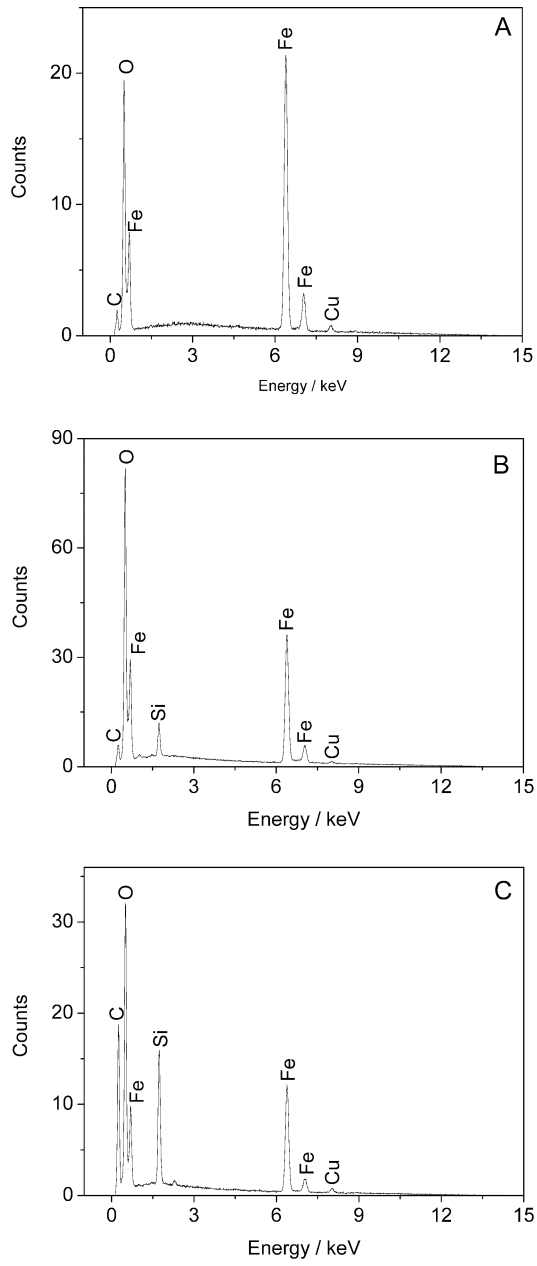

Figure 5. EDX patterns of Fe_3O_4 nanospheres (A), $Fe_3O_4-NH_2$ (B), and magnetic MIPs (C).

The above results confirmed that MIPs were coated onto the Fe_3O_4 nanospheres.

The X-ray power diffraction of the bare and MIPs coated Fe_3O_4 nanospheres are shown in Figure 3. It indicates that Fe_3O_4 is the dominant phase in both the samples. Five characteristics peaks for Fe_3O_4, marked by their indices (220), (311), (400), (511), and (400), were observed for both samples and it revealed that the resultant particles were pure Fe_3O_4 with a spinel structure.[35] Also, the polymerized process did not cause any phase change of Fe_3O_4.

TEM and SEM images of Fe_3O_4 and magnetic MIPs are shown in Figure 4. A basic core−shell structure (dark colored core for Fe_3O_4 and light colored shell for MIPs) was obtained (b). Average thickness of the outer MIPs layer determined was about 10 nm, which was close to the special size of BHb, indicating the imprinted sites almost situated at the surface of magnetic MIPs. And the average thickness of magnetic NIPs was also about 10 nm observed by TEM.

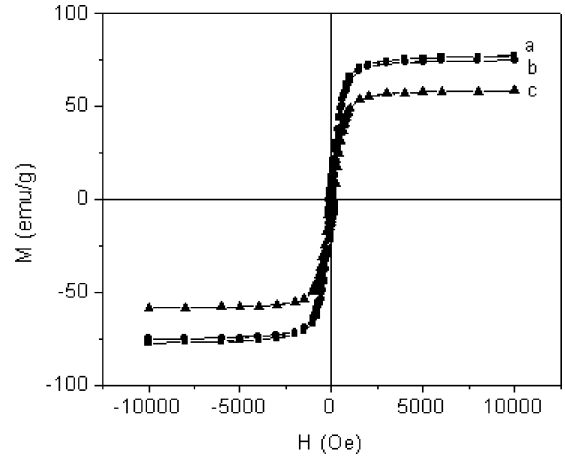

Figure 6. Magnetization curves obtained by VSM at room temperature of Fe_3O_4 nanospheres (a), $Fe_3O_4-NH_2$ (b), and magnetic MIPs (c).

Figure 5 shows the patterns of EDX measurement of the Fe_3O_4 nanopsheres (a), $Fe_3O_4-NH_2$ (b), and magnetic MIPs (c). Peaks for the elements of Fe, O, C and Cu were observed together in Figure 5a. The Cu and C peaks are due to the copper adherence tape. Besides Fe, O, C, and Cu peaks, Si peak could be found in pattern of Figure 5b, indicating γ-APS had been modified onto the Fe_3O_4 nanopsheres surface. Higher percentage of Si and C and lower percentage of Fe in pattern of Figure 5c confirmed the successfully synthesis of magnetic MIPs.

The magnetic properties of the materials were analyzed by VSM at room temperature. The magnetic hysteresis curves of the Fe_3O_4 nanopsheres, $Fe_3O_4-NH_2$, and magnetic MIPs are shown in Figure 6 as curve a, b, and c. A layer of MIPs was grafted onto the surface of Fe_3O_4 nanopsheres, so the content of magnetite in the magnetic MIPs decreased somewhat, resulting in a little lower saturation magnetization of magnetic MIPs than that of Fe_3O_4 nanopsheres. With such high saturation magnetization, the prepared polymers are very susceptible to magnetic fields and could easily and quickly be separated from a suspension, as shown in Figure 7. In the absence of an external magnetic field, a dark homogeneous dispersion existed (A). When an external magnetic field was applied, the black magnetic MIPs were attracted to the wall of vial with the solution becoming clear and transparent (B). The powerful magnetism of the magnetic MIPs could be used for a rapid magnetic separation for proteins or other biomolecules.

Adsorption Properties of Magnetic MIPs. On the basis of the result of UV−vis analysis, the amount of BHb covalent bound onto Fe_3O_4 nanospheres in the magnetic MIPs was calculated from the subtraction of BHb in supernate before and after covalent bound by Fe_3O_4-CHO nanospheres. On average, 29 mg of BHb could be immobilized on 1 g of Fe_3O_4-CHO nanospheres.

Adsorption Kinetics of the Magnetic MIPs. The adsorption kinetics of BHb was investigated by changing the adsorption time from 0 to 150 min and the initial concentration of BHb was kept constantly at 0.4 mg/mL. A curve-fitted using a sigmoid curve of the dynamic adsorption was presented in Figure 8. This is the typical kinetic curve for most rebinding processes, revealing a rapid dynamic adsorption of BHb to the magnetic MIPs. At first 1 h, absorption amounts increased with the increase of adsorption time, and then the adsorption amounts kept a constant in the afterward time. The thickness of MIPs layer grafted onto Fe_3O_4 nanosphere surface is about 10 nm, which closed to the spatial size of imprinting protein (BHb, $5.5 \times 5.5 \times 7.0$ nm). Therefore, most of imprinted cavities were

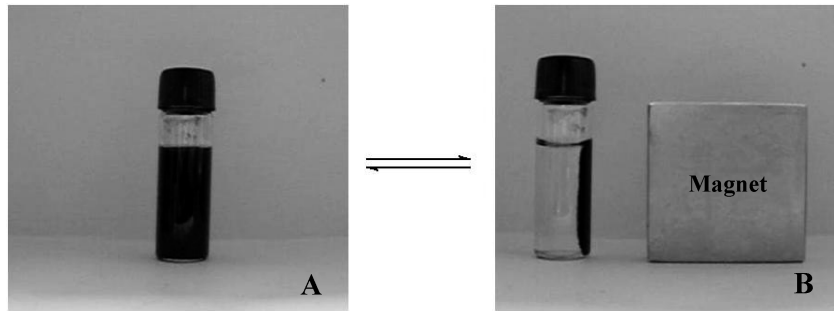

Figure 7. Photographs of magnetic MIPs without external magnetic field (A), and with external magnetic field (B). A color change from black to transparent is observed when an external magnetic field is used.

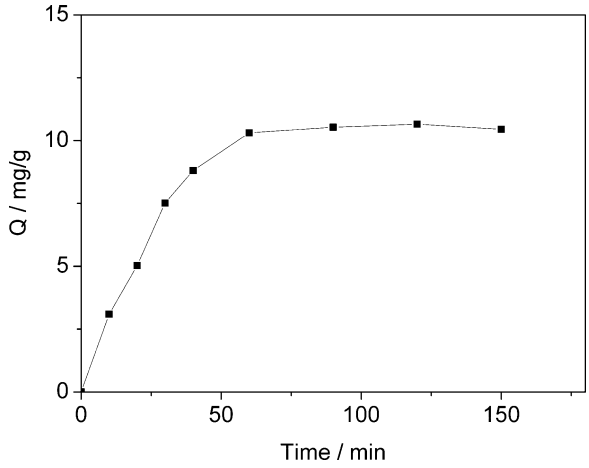

Figure 8. Adsorption dynamic curves of magnetic MIPs. Amount of polymer: 20 mg. Volume: 2.0 mL.

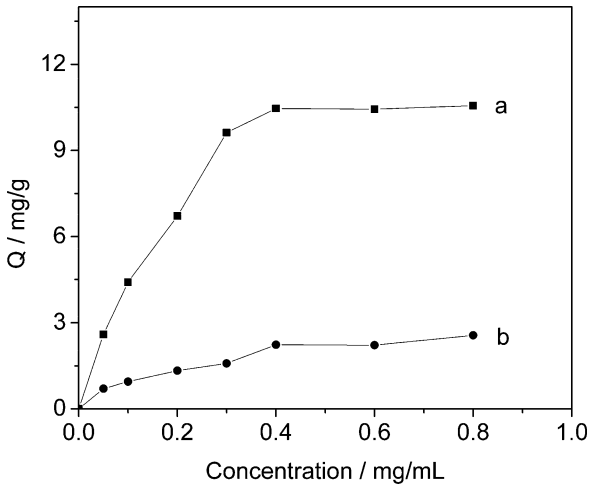

Figure 9. Adsorption isotherms of BHb on magnetic MIPs (a), and on magnetic NIPs (b). Amount of polymers: 20 mg. Volume: 2.0 mL.

situated at the surface of the magnetic MIPs, which makes the recognition sites accessible for the template molecules and thus take shorter time to gain adsorption equilibrium.

Adsorption Isotherms of the Magnetic MIPs. The method generally used to study the thermodynamic adsorption properties of MIPs is to plot an adsorption isotherm curve. Figure 9 shows an adsorption isotherm of magnetic MIPs and NIPs. The results showed that the magnetic MIPs had higher binding capacity for BHb than that of the magnetic NIPs. When initial concentration of BHb is 0.4 mg/mL, the amounts of BHb adsorbed were calculated to be 10.52 mg/g toward magnetic MIPs and 2.28 mg/g toward magnetic NIPs, respectively. The imprinting factor

TABLE 1: Effect of pH on BHb Adsorption Amounts on Magnetic MIPs and Magnetic NIPs

pH	ζ potential (mV)	Q for magnetic MIPs (mg/g)	Q for magnetic NIPs (mg/g)
6.5	−9.6	10.52	2.28
7.0	−11.0	1.72	2.53
7.4	−15.5	1.52	1.71

(the amounts of BHb bound by magnetic MIPs/the amounts of BHb bound by magnetic NIPs) was 4.6, which indicated that the prepared magnetic MIPs had high specific adsorption for the template protein.

The effect of the pH on the adsorption of the template protein was studies for magnetic MIPs and NIPs in the presence of 0.4 mg/mL BHb. Their ability for the adsorption of the template protein at pH 6.5, 7.0, and 7.4 were measured and the affinities of the polymers to BHb were assessed for each buffer, as shown in Table 1. It is clearly indicated that the adsorption amount of BHb onto magnetic MIPs depended significantly on the pH other than magnetic NIPs. Adsorption capacity value of magnetic MIPs varied from 1.25 mg/g at pH 7.4 to 10.52 mg/g at pH 6.5, while the adsorption capacity value of magnetic NIPs changed a little. For magnetic MIPs, the results showed that there was the strongest adsorption at pH 6.5, owing to the electrostatic interactions between the polymers and BHb. The ζ-potential is overall charge a particle acquires in a specific medium. ζ-potentials of magnetic MIPs and NIPs were negative (−6.9, −11.0,−15.5 mV) under experimental conditions (pH 6.5, 7.0, and 7.4, respectively), as shown in Table 1. As expected, the electrostatic interactions between negative polymers and positive BHb (pI 6.8) enhanced the adsorption at pH 6.5 while the electrostatics interactions between them were repulsive force under the other two pHs. By comparing magnetic MIPs and NIPs, the optimum pH for a binding assay was assessed at about pH 6.5, due to the fact that the affinity displayed by BHb for magnetic MIPs was several times higher than that for magnetic NIPs. Therefore, the incubation buffer of pH 6.5 was adopted in the subsequent selective adsorption experiments.

Selectively of Magnetic MIPs. The special selectivity test of magnetic MIPs was carried out using BSA (M_w 68 kDa, pI 4.6), Lyz (M_w 14.4 kDa, pI 10.7), Rnase A (M_w 13.7 kDa, pI 4.58), and EA (M_w 42.7 kDa, pI 4.7) as comparative substrates. The amounts of adsorption of these proteins to magnetic MIPs and NIPs were determined with the equilibrium adsorption method, as shown in Figure 10.

The selected comparative proteins possess large differences in molecular mass and isoelectric points (pI). The results showed that the amounts of template protein adsorbed on polymers were more than those of comparative proteins. Moreover, except for Lyz, the adsorbed amounts of the other proteins did not have

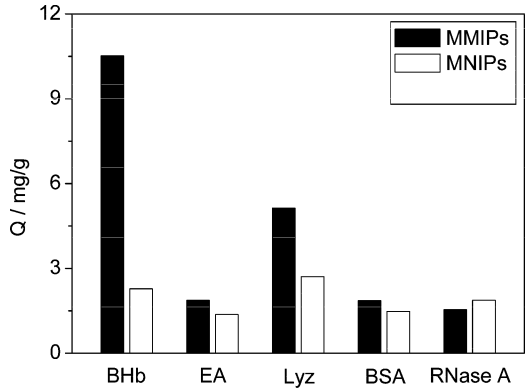

Figure 10. Selectivity of magnetic MIPs.

evident differences between magnetic MIPs and NIPs. At pH 6.5, the polymers with net negative charge repulsed comparative proteins with negative charge, such as BSA (pI 4.6),EA (pI 4.7), and RNase A (pI 4.58), resulting in the lower adsorption. However, Lyz exhibited higher adsorptions toward magnetic MIPs and NIPs than those of other comparative proteins. On the one hand, positive Lyz (pI 11.1) (under pH 6.5) should be adsorbed more readily on the surface of negatively charged polymers. On the other hand, Lyz, with smaller mass (M_w 14, 500; 4.5 × 3.0 × 3.0 nm), was easier to diffuse into the imprinting cavities and caused some nonspecific adsorption. The larger proteins were easier to be excluded from the binding cavities due to sterics, which led to low adsorption capacity. The results illuminated that the imprinting polymers were able to selectively adsorb template protein. Except for the size, shape, and position of recognition functional groups complementary between BHb and the binding sites in magnetic MIPs, the electrostatic interaction also was the main reason for recognizing the imprinting molecule.

Conclusion

Recognition of BHb, by forming molecularly imprinted materials on Fe_3O_4 nanospheres surface and combining recognition with magnetic responsible capacity, was shown to be a viable method for distinguishing the template protein from a varied selection of comparative proteins. Magnetic responsive experimental results showed that the magnetic MIPs could be easily and quickly separated from the suspension under a magnetic field, leading to a high attraction for separation of proteins or other biomolecules. Because the thickness of outer MIPs layer was approximate to the size of BHb, magnetic MIPs exhibited the fast adsorption dynamics, excellent special adsorption and recognition capacity to BHb. The spatial size complementary and electrostatic interaction between BHb and the binding sites in magnetic MIPs were the main reasons for the recognition capacity of prepared polymers.

Acknowledgment. We greatly appreciate the support of the National Natural Science Foundation of China for Key Program (20535020, 20635020, 20721002, and 9081302) and the National Basic Research Program of China (2007CB925102).

References and Notes

(1) Wulff, G.; Sarhan, A.; Zabrocki, K. *Tetrahedron Lett.* **1973**, *44*, 4329.
(2) Vlatkis, G.; Andersson, L. L.; Muller, R.; Mosbach, K. *Nature* **1993**, *361*, 645.
(3) Xie, C.; Liu, B.; Wang, Z.; Gao, D.; Guan, G.; Zhang, Z. *Anal. Chem.* **2008**, *80*, 437.
(4) Espinosa-Garcia, B. M.; Argueelles-Monal, W. M.; Hernandez, J.; Felix-Valenzuela, L.; Acosta, N.; Goycoolea, F. M. *Biomacromolecules* **2007**, *8*, 3355.
(5) Emgenbroich, M.; Borrelli, C.; Shinde, S.; Lazraq, I.; Vilela, F.; Hall, A. J.; Oxelbark, J.; De Lorenzi, E.; Courtois, J.; Simanova, A.; Verhage, J.; Irgum, K.; Karim, K.; Sellergren, B. *Chem.−Eur. J.* **2008**, *14*, 9516.
(6) Kan, X.; Zhao, Y.; Geng, Z.; Wang, Z.; Zhu, J.-J. *J. Phys. Chem. C* **2008**, *112*, 4849.
(7) Fang, G.-Z.; Tan, J.; Yan, X.-P. *Anal. Chem.* **2005**, *77*, 1734.
(8) Yoshida, M.; Hatate, Y.; Uezu, K.; Goto, M.; Furusaki, S. *Colloids Surf., A* **2000**, *169*, 259.
(9) Southard, G. E.; Houten, K. A. V.; Murray, G. M. *Macromolecules* **2007**, *40*, 1395.
(10) Reimhult, K.; Yoshimatsu, K.; Risveden, K.; Chen, S.; Ye, L.; Krozer, A. *Biosens. Bioelectron.* **2008**, *23*, 1908.
(11) Bolisay, L. D.; Culver, J. N.; Kofinas, P. *Biomacromolecules* **2007**, *8*, 3893.
(12) Sambe, H.; Hoshina, K.; Moaddel, R.; Wainer, I. W.; Haginaka, J. *J. Chromatogr., A* **2006**, *1134*, 88.
(13) Liu, X.; Chen, Z.; Zhao, R.; Shangguan, D.; Liu, G.; Chen, Y. *Talanta* **2007**, *71*, 1205.
(14) Chuang, S.-W.; Rick, J.; Chou, T.-C. *Biosens. Bioelectron.* **2009**, *24*, 3170.
(15) Gong, C.; Michael, H. L.; Yu, H. *Adv. Funct. Mater.* **2006**, *16*, 1759.
(16) Wei, S.; Molinelli, A.; Mizaikoff, B. *Biosens. Bioelectron.* **2006**, *21*, 1943.
(17) Bossi, A.; Bonini, F.; Turner, A. P. F.; Piletsky, S. A. *Biosens. Bioelectron.* **2007**, *22*, 1131.
(18) Kim, T. H.; Ki, C. D.; Cho, H.; Chang, T.; Chang, J. Y. *Macromolecules* **2005**, *38*, 6423.
(19) Cater, S. R.; Rimmer, S. *Adv. Funct. Mater.* **2004**, *14*, 553.
(20) Duy, S. V.; Lefebvre-Tournier, I.; Pichon, V.; Hugon-Chapuis, F.; Puy, J.-Y.; Périgaud, C. *J. Chromatogr. B* **2009**, *877*, 1101.
(21) Qin, L.; He, X.-W.; Zhang, W.; Li, W.-Y.; Zhang, Y.-K. *Anal. Chem.* **2009**, *81*, 7206.
(22) Hua, Z. D.; Chen, Z. Y.; Li, Y. Z.; Zhao, M. P. *Langmuir* **2008**, *24*, 5773.
(23) Titirici, M. M.; Sellergren, B. *Chem. Mater.* **2006**, *18*, 1773.
(24) Gao, D.; Zhang, Z.; Wu, M.; Xie, C.; Guan, G.; Wang, D. *J. Am. Chem. Soc.* **2007**, *129*, 7859.
(25) Niu, J.; Liu, Z.; Fu, L.; Shi, F.; Ma, H.; Ozaki, Y.; Zhang, X. *Langmuir* **2008**, *24*, 11988.
(26) Kim, T. H.; Ki, C. D.; Cho, H.; Chang, T.; Chang, J. Y. *Macromolecules* **2005**, *38*, 6423.
(27) Cater, S. R.; Rimmer, S. *Adv. Funct. Mater.* **2004**, *14*, 553.
(28) Gao, B.; Wang, J.; An, F.; Liu, Q. *Polymer* **2008**, *49*, 1230.
(29) Titirici, M. M.; Sellergren, B. *Chem. Mater.* **2006**, *18*, 1773.
(30) Menaker, A.; Syritski, V.; Reut, J.; Öpik, A.; Horváth, V.; Gyurcsányi, R. E. *Adv. Mater.* **2009**, *21*, 2271.
(31) Linares, A. V.; Vandevelde, F.; Pantigny, J.; Falcimaigne-Cordin, A.; Haupt, K. *Adv. Funct. Mater.* **2009**, *19*, 1299.
(32) Tan, C. J.; Tong, Y. W. *Anal. Chem.* **2007**, *79*, 299.
(33) Li, Y.; Yin, X.-F.; Chen, F.-R.; Yang, H.-H.; Zhuang, Z.-X.; Wang, X.-R. *Macromolecules* **2006**, *39*, 4497.
(34) Li, L.; He, X.; Chen, L.; Zhang, K. *Chem. Asian. J.* **2009**, *4*, 286.
(35) Deng, H.; Li, X.; Peng, Q.; Wang, X.; Chen, J.; Li, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 2782.
(36) Asem, A. A.; Ahmed, M. D.; Waheeba, A. A.-A. *Chem. Eng. J.* **2009**, *150*, 55.
(37) Behforouz, M.; Cai, W.; Mohammadi, F.; Stochsdale, M. G.; Gu, Z.; Ahmadian, M.; Baty, E.; Etling, M. R.; Anzi, C. H.; Swiftney, T. M.; Tanzer, L. R.; Merriman, R. L.; Behforouz, N. C. *Bioorg. Med. Chem.* **2007**, *15*, 495.
(38) Sun, Y.; Zhang, Z.; Wong, C. P. *J. Colloid Interface Sci.* **2005**, *292*, 436.
(39) Bao, L. R.; Yee, A. F. *Polymer* **2002**, *43*, 3987.
(40) Zhang, C.; Wängler, B.; Morgenstern, B.; Zentgraf, H.; Eisenhut, M.; Untenecker, H.; Krüger, R.; Huss, R.; Seliger, C.; Semmler, W.; Kiessling, F. *Langmuir* **2007**, *23*, 1427.
(41) Li, G.-S.; Li, L.-P.; Smith, J. R. L.; Inomata, H. *J. Mol. Struct.* **2001**, *560*, 87.

JP910060C