Preparation of Molecularly Imprinted Polymers Functionalized with Core–shell Magnetic Nanoparticles for the Recognition of Glycoprotein

He-Ye WANG[a], Li LI[b], Yong-Fu ZHAO[c,*]

Institute of Facilities and Equipment in Agriculture, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu 210014, P. R. China

[a]hywang790907@163.com, [b]muzishuiqudou@163.com, [c]zyfzyf2002@163.com

*Corresponding author

Keywords: Molecularly Imprinted Polymers, Core–shell Magnetic Nanoparticles, Glycoprotein.

Abstract. A method to prepare molecularly imprinted polymers (MIPs) coatings on magnetic Fe_3O_4 nanoparticles (MNPs) with core–shell structure for the recognition of glycoprotein was developed. The factors that influence the Fe_3O_4@MIPs, such as polymerization time, extraction time were investigated.

Introduction

Molecularly imprinted polymers (MIPs) are artificial, template-made receptors with the ability to recognize and to specially bind the target molecule [1-2]. The stability, ease of preparation and low cost of these materials have led to their assessment as substitutes for antibodies or enzymes in chemical sensors, catalysis and separations.

Glycoproteins, which occupy more than 50% of the total proteins in mammalian systems, play key roles in many biological processes, such as molecular recognition, inter- and intra-cellular signaling, and immune response. Besides, many glycoproteins are disease biomarkers and therapeutic targets. Therefore, the imprinting of glycoproteins is of great importance and in high demand.

Boronic acids can covalently interact with cis-diol-containing molecules such as sugars or glycoproteins to form stable cyclic esters in an alkaline aqueous solution while the boronate esters dissociate when the environmental pH is switched to acidic [3]. This reversible binding has made boronic acids excellent affinity ligands for creating functionalized materials [4-6].

In this study, horseradish peroxidase (HRP) was selected as a glycoprotein model. Boronic acid-functionalized magnetic Fe_3O_4 nanoparticles (MNPs) were synthesized for immobilized templates, and then Fe_3O_4@MIPs MNPs were prepared.

Materials and Methods

Materials and Reagents

Tetraethoxysilane (TEOS) and 3-aminopropyltriethoxylsilane (APTES) were purchased from Alfa Aesar Chemical Company (Tianjin, China). Horseradish peroxidase (HRP), acrylamide(AM), N,N-methylene-bis-(acrylamide) (Bis), ammonium persulfate (APS) N,N,N',N'-tetramethylethylenediamine (TEMED) and 3-aminophenylboronic acid (APBA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ferric trichloride hexahydrate, ethylene glycol, anhydrous sodium acetate 1, 6-hexanediamine and glutaraldehyde (GA) were from Sinopharm Chemical Reagent (Shanghai, China). All reagents used were of analytical grade or higher. All commercially available reagents were used without further purification. Water used in all the experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA, USA).

Preparation of the Core–shell Structure Fe_3O_4@MIPs and Non-imprinting Polymer (NIPs) MNPs

The synthesis procedure of Fe_3O_4@SiO_2@MIPs MNPs was as shown in Fig. 1.

(1) Synthesis of amino functionalized MNPs. Amino functionalized MNPs were first synthesized

by the solvent-thermal method [7] with minor modification. Briefly, 2.0 g ferric trichloride hexahydrate, 13.0 g 1, 6-hexanediamine and 4.0 g anhydrous sodium acetate were mixed with 60 mL glycol in a PTFE-lined autoclave and reacted at 198 ℃ for 6 h. The resulting MNPs were rinsed with water and ethanol for 3 times each and then dried at 50 ℃.

(2) Preparation of silica shell. To cover the amino functionalized MNPs with a silica shell, 2.7 mL TEOS and 15.0 mL ammonium hydroxide were added in 400 mL ethanol and then the mixture was left to react at room temperature for 20 min (20 ℃). Then 250 mg MNPs were added and the reaction was left for another 20 min. The Fe_3O_4@SiO_2 MNPs were collected by a magnet at the wall and washed 3 times with ethanol and dried at 50 ℃.

(3) Functionalized with boronic acids. 250 mg Fe_3O_4@SiO_2 MNPs were dispersed in 40 mL anhydrous toluene containing 2 mL APTES, the mixture was mechanically stirred (400 rpm) for 12 h at 120 ℃. The resultant APTES-functionalized MNPs washed 3 times with ethanol and dried at 50 ◦C. 200 mg APTES-functionalized MNPs was dispersed in 40 mL 5% glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.0) and the mixture was mechanically stirred for 2 h. The glutaraldehyde-activated MNPs were cleaned by 100 mM sodium phosphate buffer three times then dispersed in 40 mL 5 mg/mL 3-aminophenylboronic acid 100 mM sodium phosphate buffer which contained 1% (w/w) sodium cyanoborohydride. After 2 h reaction, the resultant MNPs were magnetically collected and washed with water and alcohol, dried at 50 ℃, and stored for further use.

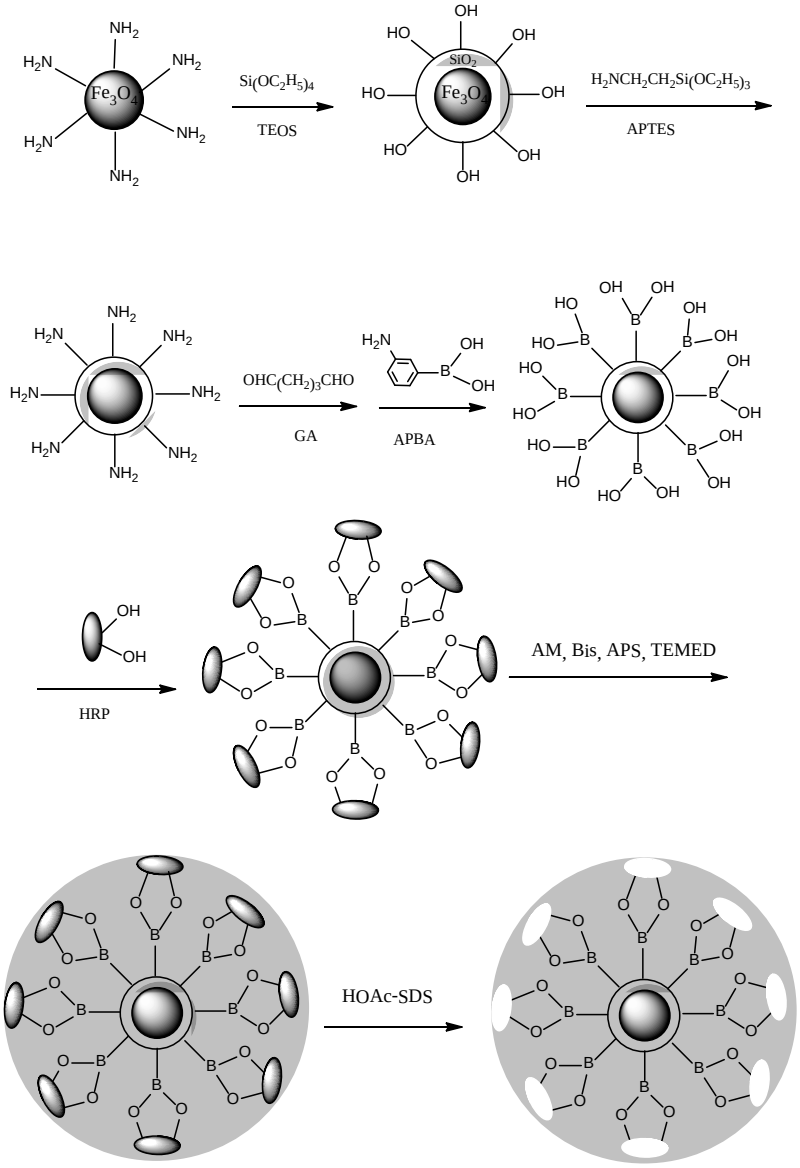

Fig.1 Schematic of preparation of molecularly imprinted polymers functionalized with core–shell magnetic nanoparticles for glycoprotein

(4) Preparation of the core–shell structure Fe_3O_4@MIPs and Fe_3O_4@NIPs MNPs. HRP was chosen as templates and boronic acid-functionalized MNPs as cores. The functional monomer, cross-linking agent, initiator and accelerator used in this study are acrylamide (AM), N,N-methylene-bis-(acrylamide) (Bis), ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED), respectively. 10% HOAc-10% sodium dodecyl sulfate (SDS) was then added to remove the template proteins.

Non-imprinted Fe_3O_4@NIPs MNPs was prepared following the same procedure in the absence of the template protein.

Result and Discussion

Characterization of Fe_3O_4@MIPs MNPs

The size and morphology of the Fe_3O_4@MIPs MNPs were characterized by TEM. As shown in Fig.2, TEM images suggest that the MNPs were well shaped with a diameter of about 100 nm. Because TEM fails to recognize the Fe_3O_4 core, the SiO_2 shell and the MIPs layer, the thickness of the SiO_2 shell and the MIPs coating is unknown.

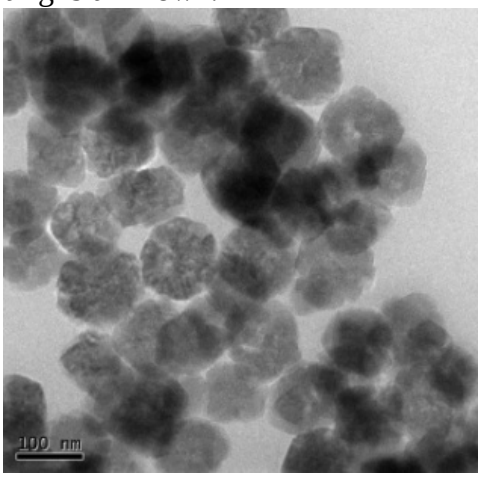

Fig.2 TEM images for Fe_3O_4@MIPs MNPs

Effect of the Polymerization Time

Polymerization time is an important factor that determines the Fe_3O_4@MIPs MNPs. In this study, four time points, including 12, 15, 18 and 21 h, were compared under otherwise identical conditions. As shown in Fig.3, 15 h provided the most intense signal for HRP extraction. HRP extraction amount by Fe_3O_4@MIPs MNPs is greater than that of Fe_3O_4@NIPs MNPs. HRP extracted was detected with chemiluminescence method according to a literature [8].

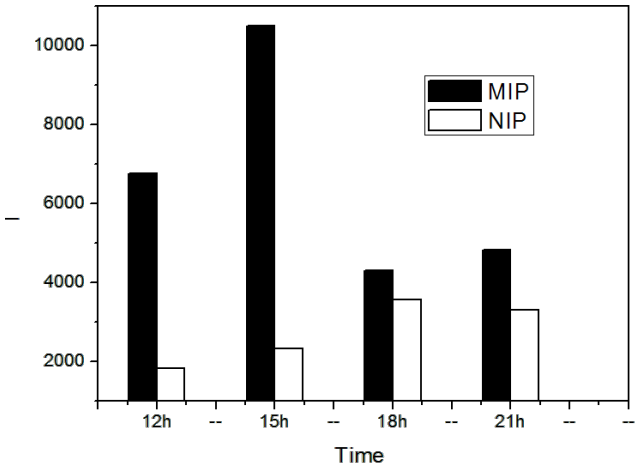

Fig.3 Effect of the polymerization time

Effect of the Extraction Time

Four different extraction time points with Fe_3O_4@MIPs MNPs and Fe_3O_4@NIPs MNPs were studied. As shown in Fig.4, 2.5 h provided the most intense signal, which suggests that longer extraction time favors the extraction efficiency.

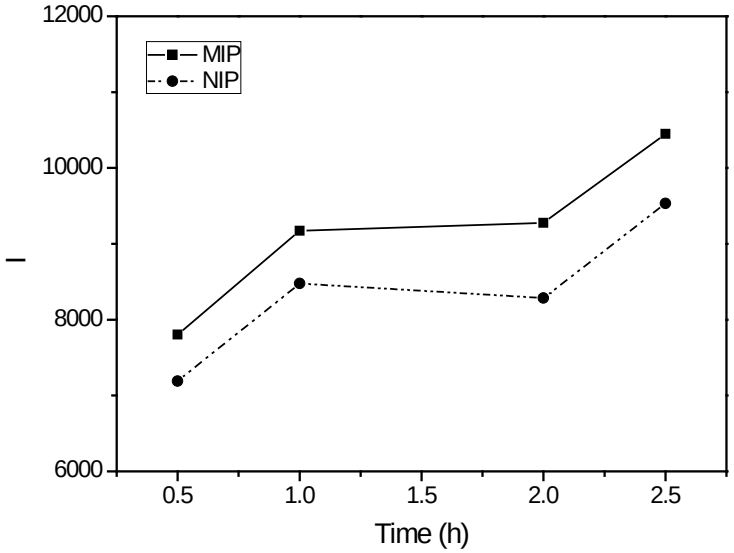

Fig.4 Effect of the extraction time

Conclusion

In this study, by combining boronate affinity interaction and molecular imprinting, a method was developed to prepare MIP coatings on magnetic Fe_3O_4 MNPs with core-shell structure for the recognition of glycoprotein. The factors that influence the Fe_3O_4@MIPs, such as polymerization time, extraction time were studied.

Acknowledgements

This research was financially supported by Special Fund for Agro-scientific Research in the Public Interest (201103007).

Reference

[1]K. Haupt, K. Mosbach, Molecularly imprinted polymers and their use in biomimetic sensors, Chem. Rev. 100 (2000) 2495-2504.

[2]L. Ye, K. Mosbach, Molecular imprinting: synthetic materials as substitutes for biological antibodies and receptors, Chem. Mater. 20 (2008) 859-868.

[3]T.D. James, K.R.A.S. Sandanayake, S. Shinkai, Saccharide sensing with molecular receptors based on boronic acid, Angew. Chem. Int. Ed. Engl. 35 (1996) 1910-1922.

[4]L.B. Ren, Z. Liu, Y.C. Liu, P. Dou, H.Y. Chen, Ring-opening polymerization with synergistic co-monomers: access to a boronate-functionalized polymeric monolith for the specific capture of cis-diol-containing biomolecules under neutral conditions, Angew. Chem. Int. Ed. 48 (2009) 6704–6707.

[5]H.Y. Li, H.Y. Wang, Y.C. Liu, Z. Liu, benzoboroxole-functionalized monolithic column for the selective enrichment and separation of cis-diol containing biomolecules, Chem. Commun. 48 (2012) 4115-4117.

[6]H.Y. Wang, Z.J. Bie, C.C. Lü, Z. Liu, Magnetic nanoparticles with dendrimer-assisted boronate avidity for the selective enrichment of trace glycoproteins, Chem. Sci. 4 (2013) 4298-4303.

[7]L. Y. Wang, J. Bao, L.Wang, F. Zhang, Y. D. Li, One-potsynthesis and bioapplication of amine-functionalized magnetite nanoparticlesand hollow nanospheres, Chem. Eur. J. 12 (2006) 6341-6347.

[8]L. Li, Y. Lu, Z.J. Bie, H.Y. Chen, Z. Liu, Photolithographic boronate affinity molecular imprinting: a general and facile approach for glycoprotein imprinting, Angew. Chem. Int. Ed. 52 (2013) 7451-7454.