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Rapid and efficient separation of glycoprotein using pH double-responsive imprinted magnetic microsphere

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ABSTRACT

As biomarkers of many diseases, glycoproteins are of great significance to clinical diagnostics. However, the determination of low abundant glycoproteins in complex biological samples without any pretreatment process is still a problem. In this study, a rapid and convenient separation method for highly efficient enrichment of glycoproteins is reported, based on pH double-responsive imprinted magnetic microspheres. Thin imprinted polymer shells were fabricated onto the surface of magnetic microspheres by free radical polymerization, using 2-(Dimethylamino) ethyl methacrylate as pH-sensitive monomer, 4-vinylphenylbronic acid as boronate affinity monomer, and ovalbumin (OVA) as template molecule. Combining the advantages of pH-sensitive monomer and boronate affinity monomer, rapidly capture-release of OVA could be modulated by changing solution pH. Moreover, high absorption ability (81.2 mg/g) was achieved within about 10 min. This study provided responsible way to imprint glycoproteins and showed great potential for glycoprotein detection in clinical diagnostic.

1. Introduction

Glycoproteins which contain glycans covalently attached to polypeptide side-chains play key roles in numerous biological processes, such as cell–cell interaction, molecular recognition and immune response [1]. Moreover, they have been treated as therapeutic targets and disease biomarkers, because of their close association with the occurrence of diseases, especially cancer and inflammation [2,3]. Therefore, the research of glycoprotein is of great importance. Mass spectrometry has been proven to be the main methods for the glycoprotein detection. However, the sensitivity and accuracy are commonly limited, owing to the complexity of matrix and low abundance of glycoproteins (2–5%) [4,5]. Thus, a convenient and efficient approach to separation and enrichment of glycoproteins is in high demand.

Currently, the main methods to separate glycoproteins are as follow: lectins [6], hydrazide chemistry [7] and boronate affinity chromatography [8,9]. Boronate affinity materials have attracted increasing attention and found plenty of application in recent years. Boronic acid can covalently interact with cis-diol-containing molecules including nucleosides, glycans, glycopeptides and glycoproteins and so on. The unique feature is that they are capable of forming covalent complexes with cis-diol-containing molecules at comparatively high pH, while the complexes dissociate reversibly in an acidic environment [10]. Compared with lectins and hydrazide chemistry, boronate affinity chromatography shows many attractive characteristics, including pH-controlled reversible binding, good compatibility and rapid adsorption kinetics [11].

However, the boronate affinity materials commonly have the shortcomings of nonspecific adsorption of non glycoprotein. Motivated by such concept, the combination of boronate affinity materials and molecular imprinting comes into being [12,13]. It is worth mentioning that, with the boronic acid as the functional monomers, the molecularly imprinted polymers (MIPs) could exhibit several significant advantages, such as high affinity, reversible binding and excellent tolerance [14]. Molecular imprinting is an inexpensive and high selectivity technique, which produce template-shape cavities in matrices with memory of the target molecule, thus attracts great attention on the fields of purification [15], selective separation [16–18], and sensor fabrication [19,20]. Nevertheless, the imprinting of biomacromolecules is still a big challenge, because of their relatively large sizes, leading to the difficulties in target molecules removal and rebinding [21]. Surface molecular imprinting, a technology that designing the polymerization reaction proceeded in the polymeric materials surface, has proved to be sufficient to fabricate imprinted sites and minimize the mass transfer resistance.

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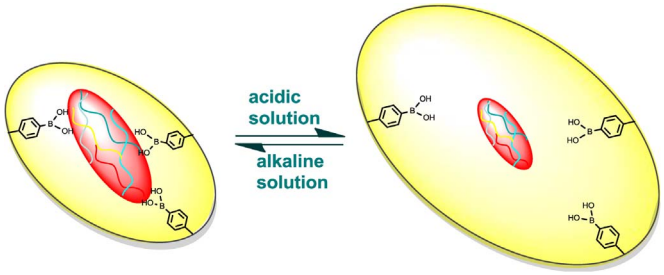

Scheme 1. The synergistic effect of pH-responsive hydrogels and boronate affinity: swelling/shrinking imprinted cavities and reversible boronate affinity bond on the change in the pH.

Meanwhile, stimuli-responsive materials which exhibit volume phase transition under the stimuli of circumstances (e.g., magnetic, temperature, pH, photo and salt), are also an alternative to solve the problem of limited mass transfer. The stimuli-responsiveness is based on the change in the hydrophilicity of polymer networks. The smart materials have great potential applications, such as drug delivery systems [22,23], sensors [24] and separation technology [25,26]. The pH-responsive hydrogels fall into two types in accordance with ionizable groups: anionic and cationic [27]. To date, there have been a few researches on pH-responsive hydrogels MIP [28–30]. However, as far as we know, the pH-responsive hydrogels have not been employed on MIPs for glycoproteins.

In this study, we presented novel magnetic MIPs nanoparticles combining the advantage of boronate affinity and cationic pH-responsive hydrogels to selectively separate glycoproteins. The covalent bond between target molecules and functional monomer could dissociate and the memory cavities could get bigger, when the environment pH was shifted to acidic (Scheme 1). 4-vinylphenylbronic acid (VPBA), 2-(Dimethylamino) ethyl methacrylate (DMA) and N, N-methylenebis acrylamide (MBA) were selected to frame the MIP layer. The pH-sensitive swelling/shrinking behavior was investigated by measuring swelling radio at different pH. The adsorption property of the resultant surface MIPs was demonstrated by rebinding experiments. It was found that, the prepared MIPs showed excellent adsorption ability and elution efficiency, compared with traditional molecular imprinting.

2. Experimental section

2.1. Materials and instruments

Ovalbumin (OVA, pI=4.7, MW=45.0 kDa) was supplied from Sigma-Aldrich Co. (St Louis, MO, USA). Horseradish peroxidase (HRP, pI=7.2, MW=44 kDa) and albumin Bovine (BSA, pI=4.7, MW=66.4 kDa) were purchased from Shanghai Sangon Co., Ltd. (Shanghai, China). Transferrin (TRF, pI=5.5, MW=80.0 kDa,) was obtained from Solarbio Co. (Beijing, China). 4-vinylphenylbronic acid (VPBA, 98%) and 2-(Dimethylamino) ethyl methacrylate (DMA, 99.5%) were obtained from Energy Chemical. Ammonium persulfate (APS) and N, N-methylenebis acrylamide (MBA, 99.0%) were obtained from Tianjin Kermel Chemical Reagent Co., Ltd. Vinyltriethoxysilane (VTEO, 97%) was purchased from Aladdin. Other chemicals were all of analytical grade and used as received.

Fourier transform infrared (FTIR) spectra were recorded on a NICOLET 380 spectrophotometer in KBr medium. The morphologies and structures of the microspheres were investigated by a Scanning Electron Microscope (SEM, JSM-6610LV). Thermogravimetric Analysis (TGA) was carried out for the microspheres samples (5 mg) using a TGA 50 thermogravimetric analyzer under nitrogen atmosphere with a heating rate of 20 °C/min up to 800 °C. The fluorescence measurements were performed on a Shimadzu RF-5301PC spectrofluorimeter (λex=291 nm, ex/em slits=3/3 nm).

2.2. Preparation of imprinted and nonimprinted magnetic nanoparticles

Fe_3O_4@VTEO nanoparticles were synthesized based on the stöber method [31]. Briefly, the dry Fe_3O_4 (100.0 mg) nanoparticles were added in the solution prepared from 20 mL of ethanol, 5 mL of deionized water, and 0.7 mL of ammonia (28 wt%) and mixed thoroughly by ultrasonic. After 15 min stirring, 0.5 mL of vinyltriethoxysilane was injected into the solution. Subsequently, the obtained mixture was refluxed at 40 °C for 24 h. The nanoparticles were washed with ethanol to remove the unreacted component and by-product.

Synthesis of Fe_3O_4@MIPs followed the procedure below: Fe_3O_4@VTEO (25 mg), MBA (15 mg), VPBA (10 mg), DMA (60 μL), and 10 mg of OVA were dissolved in 25 mL of deionized water by ultrasonication. Then, the resultant solution was incubated 1 h under stirring for pre-polymerization and the solution was deaerated by N_2 for 1 h. Subsequently, by adding 5 mg of APS, the polymerization was initiated and continued under stirring at 25 °C for 24 h. The obtained Fe_3O_4@MIPs were washed successively with PB (20 mM, pH=2.0). The Fe_3O_4@NIPs were prepared using the same procedure but without addition of model target.

2.3. Measurement of equilibrium swelling ratio

To prove the pH-sensitive properties of Fe_3O_4@MIPs, swelling radios at different pH were investigated, the typical procedure was as follows: certain concentration of Fe_3O_4@MIPs in phosphate buffer (20 mM, pH=7.0) was dispersed by ultrasonication, then, injected the solution into the NMR tube. When it reached the same static fluid level, we could record the height, H_0. After magnetic separation, the supernatant was discarded. Subsequently, the particles were incubated in PB (20 mM, pH=2, 3, 4, 5, 6, 7, 8, respectively) for 3 h at 25 °C. After equilibrium, the resultant fluid level (H_w) was measured. The swelling radio (SR) was calculated from the following equation:

$$SR = \frac{H_w - H_0}{H_0}$$

2.4. Protein adsorption experiments

The adsorption capacity (Q) of Fe_3O_4@MIPs and Fe_3O_4@NIPs was investigated as follows: 5 mg of Fe_3O_4@MIPs and Fe_3O_4@NIPs were dispersed in 5 mL of the OVA solutions at different initial concentrations prepared in phosphate buffer (20 mM, pH=7.0). The obtained mixtures were shaking at 25 °C for 30 min. Finally, the supernatants were determined. The amount of OVA adsorbed by Fe_3O_4@MIPs/Fe_3O_4@NIPs was calculated as:

$$Q = \frac{(c_0 - c)V}{m}$$

In this equation, c_0 (mg/mL) and c (mg/mL) are the initial and final concentrations of the OVA sample, respectively, V (mL) is the volume of the OVA solution, and m (mg) is the dry mass of Fe_3O_4@MIP/Fe_3O_4@NIP.

2.5. Interference experiment

Three proteins differing in isoelectric points (pI) and molecular weights (MW) were chosen to investigate the selectivity of the Fe_3O_4@MIPs. The proteins with different pI and MW were TRF (pI=5.5, MW=80.0 kDa), HRP (pI=7.2, MW=44 kDa), BSA (pI=4.7, MW=66.4 kDa) 7.0 phosphate buffer solutions at a feed concentration of 0.6 mg/mL.

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