Colloids and Surfaces A: Physicochem. Eng. Aspects 433 (2013) 191–199

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Smart surface imprinting polymer nanospheres for selective recognition and separation of glycoprotein

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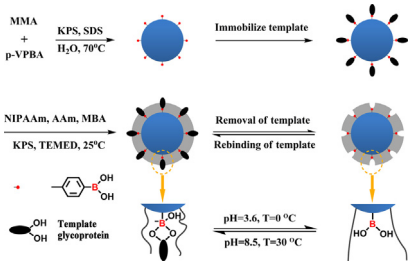
HIGHLIGHTS

- Surface imprinting nanosphere was prepared using covalent template immobilization.
- The imprinted nanospheres could rebind the template glycoprotein.
- The rebinding affinity of the imprinted nanospheres was thermo- and pH-dependent.
- High adsorption, fast rebinding kinetics, excellent selectivity and reusability.
- The applicability was favorable in a real sample analysis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 February 2013
Received in revised form 27 April 2013
Accepted 3 May 2013
Available online xxx

Keywords:
Molecular imprinting
Surface imprinting
Covalent immobilization
Nanosphere
Ovalbumin

ABSTRACT

Through covalent immobilization of template and surface imprinting, smart molecularly imprinted polymer nanospheres were developed for selective separation of the glycoprotein ovalbumin (OB). First, a boronic acid group-bearing poly (methyl methacrylate) (b-PMMA) nanosphere was synthesized directly at the high temperature of 70 °C. The b-PMMA nanosphere could pre-immobilize the template OB on its surface by forming reversible covalent bonds. Then the precipitation polymerization of N-isopropylacrylamide (NIPAAm) and acrylamide (AAm) readily occurred on the b-PMMA nanosphere as a core at room temperature, leading to the formation of core–shell molecular imprinting nanosphere. The experiments showed the rebinding affinity of the imprinted nanospheres was thermo- and pH-dependent. The resulting imprinted nanospheres showed high adsorption capacity and good specific recognition behavior toward the template molecule, and no obvious reusability deterioration was observed. Most notably, the imprinted nanospheres reached saturated adsorption within 20 min, indicating faster rebinding kinetics. In addition, the imprinted nanospheres were successfully applied to selectively separate the target OB from an egg white sample.

1. Introduction

Molecularly imprinted polymers (MIPs) are being increasingly used as artificial recognition material with the intrinsic affinity for pre-selected target molecules (the template) [1,2]. Compared to natural selective species like enzymes and antibodies, MIPs offer some conceivable advantages including high stability, ease of preparation, tailor-made recognition, and excellent reusability, and thus have showed tremendous application potential in biosensors, separation, catalysis and drug delivery [3–6]. Despite the ease of the conventional imprinting methodology and its associated success with the imprinting of small molecules [7], imprinting water-soluble bio-macromolecules like proteins still faces many challenges involving poor mass transfer, difficult removal of templates and low binding capacity owing to the large molecular

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http://dx.doi.org/10.1016/j.colsurfa.2013.05.018

size and the incompatibility between the fragile protein template and the imprinting conditions. To resolve these issues, various approaches have been proposed for the successful imprinting of proteins, such as surface imprinting [8,9], epitope-mediated imprinting [10,11], covalent imprinting [12], and the use of low cross-linking density hydrogels [13,14].

Typically, surface imprinting has been adopted to avoid entrapment of protein template in cross-linked polymers, which allowed the imprinted sites to be situated at the surface or in the proximity of the material's surface, ensuring the complete removal of templates, low mass-transfer resistance and easy access to the target molecules [15]. Especially, surface imprinting over nanosized sphere support materials with large specific surface area, is very appropriate for a template of bulky structure like protein. Many particles have been successfully used as supports in the surface imprinting process, such as silica nanoparticles [16], Fe_3O_4 magnetic nanoparticles [17], and quantum dots [18]. Nevertheless, the surface functionalization and modification of the above mentioned particles need to be achieved by a complex or multi-step graft polymerization. Methyl methacrylate (MMA) as a major monomer component can be used to copolymerize with functional monomers to obtain the desired functionalized poly MMA (PMMA) spheres by one pot method [19]. Moreover, PMMA spheres as solid carriers afford moderate mechanic strength and good biocompatibility [20], which makes them appropriate for protein imprinting.

Poly(N-isopropylacrylamide) (PNIPAAm), a well-known thermo-sensitive polymer with a lower critical solution temperature (LCST) around 32 °C in aqueous solution [21], shows the abrupt transitions in hydrophilic/hydrophobic properties and swelling/shrinking volumes across its LCST under thermal stimulus [22,23]. Thus, the switchable property offers the possibility of controlling molecule binding ability when environmental temperature is changed. Moreover, PNIPAAm hydrogels as matrices possess a degree of flexibility very similar to natural tissue, which ensures biocompatibility and conformational stability of the proteins. To date, there have been several works focusing on the design of protein-imprinted materials based on PNIPAAm [24–27].

Glycoproteins are associated with the incidence of many diseases in physiological processes [28]. However, without any enrichment process, the determination of these glycoproteins is severely interfered because of their low-abundance property in complex biology samples. Traditionally, the recognition and separation of glycoproteins have been done by lectin-based affinity chromatography [29], hydrazide chemistry [30] or size exclusion chromatography [31]. Unfavorably, these methodologies are time- and cost- consuming, which limits their widespread applications. Therefore, simple and cost-effective methods are in urgent demand. Recently, boronic acid or similar derivatives have been introduced for the isolation and enrichment of glycoproteins due to their tendency to form reversible boronate esters with cis-diols of glycoproteins in basic media [32–34]. More interestingly, this bond could be cleaved in acidic media to remove the target molecule. According to this principle [35–37], boronic acid, as a recognition element, would be a good choice to combine with MIP technique for the specific separation of glycoproteins.

In this paper, ovalbumin (OB) was selected as a model glycoprotein to study a smart separation method by surface imprinting and covalent immobilization of the template. Herein, monodispersed surface imprinting nanospheres were prepared by free radical polymerization method at two different temperatures. The initial polymerization was carried out at 70 °C to form homogeneous and monodispersed boronic acid group-bearing PMMA nanospheres (b-PMMA) as the cores for subsequent imprinting. Following covalent immobilization of the template, N-isopropylacrylamide (NIPAAm) and acrylamide (AAm) were polymerized onto the b-PMMA surface at 25 °C. Finally, monodisperse core–shell nanospheres with smart OB-imprinted polymer shells were obtained. The morphology and structure of the resulting imprinted nanospheres were characterized by scanning electron microscopy (SEM), dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectra and X-ray photoelectron spectroscopy (XPS). The protein recognition performances were evaluated by single-protein or competitive batch rebinding tests, rebinding kinetics study and regeneration in detail. In addition, the practical feasibility of the imprinted nanospheres was further assessed by separation of the template OB from an egg white sample.

2. Materials and methods

2.1. Materials and chemicals

All reagents used were of at least analytical grade. Methyl methacrylate (MMA) was purchased from Kemiou Chemical Reagent Co. (Tianjin, China). N-isopropylacrylamide (NIPAAm) was from Acros Organics (Morris Plains, NJ, USA). Sodium dodecyl sulfate (SDS) and 4-vinylphenylbronic acid (p-VPBA) were supplied from Sigma -Aldrich Co. (St Louis, MO, USA). Ovalbumin (OB, Mw = 46.0 kDa, pI = 4.7), transferrin (TF, Mw = 80.0 kDa, pI = 5.5), horseradish peroxidase (HRP, Mw = 40.0 kDa, pI = 7.2), bovine hemoglobin (BHB, Mw = 64.5 kDa, pI = 6.9), lysozyme (Lyz, Mw = 14.4 kDa, pI = 11.2), bovine serum albumin (BSA, Mw = 66.4 kDa, pI = 4.9), acrylamide (AAm), *N*, *N*′-methylenebisacrylamide (MBA), potassium persulfate (KPS) and N, N, N′, N′-tetramethylenediamine (TEMED) were obtained from Solarbio Co. (Beijing, China). Phosphate buffer solution (PBS, pH = 8.5, 10 mmol L^{-1}) was used as a working medium. Ultrapure Millipore water (18.2 MΩ) was used throughout this work.

2.2. Instrumentation

The morphologies and particle sizes of the nanospheres were characterized with a JSM-7500F scanning electron microscope (SEM, JEOL). The hydrodynamic diameter (D_h) of nanospheres was determined in aqueous solutions by dynamic light scattering (DLS) with a Zetasizer Nano ZS instrument using a He–Ne laser at a wavelength of 633 nm (Malvern, UK). X-ray photoelectron spectroscopy (XPS) measurements were performed with an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with ultra-high vacuum generators. Fourier transform infrared (FTIR) spectra (4000–400 cm^{-1}) in KBr were recorded using an AVATRA 360 FTIR spectrophotometer (Nicolet, USA). The solid-state ^{13}C NMR spectra were obtained with Infinityplus-400 spectrometer (Varian, USA). The data of adsorption were obtained by using UV-2450 spectrophotometer (Shimadzu, Japan). Electrophoresis for protein separation was carried out by regular SDS-PAGE with 15% polyacrylamide gel (Bio-Rad, USA).

2.3. Preparation of the core–shell MIP and NIP nanospheres

In this work, OB molecule was used as the template glycoprotein, and MIP nanospheres were synthesized by free-radical polymerization at two temperature stages. The first-step polymerization was conducted according to the method described previously with minor modification [38]. In order to obtain monodispersed PMMA spheres as the initial cores, 88 mL deionized water containing 7.5 mL MMA, 1.5 g p-VPBA, and 6.0 mg SDS was deoxygenated by nitrogen at room temperature for 30 min and heated to 70 °C under stirring, followed by addition of KPS (60 mg in 1 mL water). After 4 h polymerization, the reaction mixture was cooled to room temperature. The prepared b-PMMA colloids were purified three times by centrifugation at 10,000 rpm for 10 min. Then the imprinted shell on the b-PMMA sphere was prepared through precipitation