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A facile approach for imprinting protein on the surface of multi-walled carbon nanotubes

Ren Liu [a], Mo Sha [a], Sisi Jiang [a], Jing Luo [a,b,*], Xiaoya Liu [a,**]

[a] The Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi, Jiangsu 214122, China

[b] State Key Laboratory of Molecular Engineering of Polymers (Fudan University), Shanghai 200433, China

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ABSTRACT

This study describes a green, facile and low cost approach for imprinting protein on the surface of multi-walled carbon nanotubes (MWNTs) using papain as the template, dopamine as the functional monomer. By simply mixing MWNTs, dopamine, template protein in weak alkaline aqueous solution, a thin adherent polydopamine (PDA) film imprinted with protein was spontaneously obtained on the surface of MWNTs to produce the imprinted nanomaterials (MWNTs@MIPs). The obtained MWNTs@MIPs were characterized with Fourier transform infrared spectrometer (FT-IR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The adsorption process of the MWNTs@MIPs towards template protein was investigated in detail. The effects of the concentration of the monomer and template, polymerization time, extraction process were optimized. The prepared MWNTs@MIPs show fast binding kinetics, high binding capacity and acceptable specific recognition behavior towards template proteins. Furthermore, the stability and regeneration were also investigated, which indicated that the MWNTs@MIPs had good reusability. The good recognizing behavior coupled to the low cost and facile one-step preparation make the MWNTs@MIPs attractive for separation and specific protein recognition.

1. Introduction

Molecular imprinted polymers (MIPs), described as artificial "locks" for "molecular keys", have the ability to recognize the used template from a mixture of closely related compounds. In other words, recognition sites were generated by polymerizing functional monomer together with the template molecules in the presence of a cross-linking agent. After removing the template molecules, imprinted cavities were left inside the polymer network [1,2]. Owing to their molecular recognition ability, low cost, chemical and mechanical stability, durability, reusability and ease of preparation, MIPs can act as chemical sensors, artificial antibodies, and have been used in separations, enrichments, catalysis, and membrane filtration [3–8].

Despite the attractive features of this technique, MIPs prepared by traditionally bulk polymerization exhibited poor accessibility, low-affinity binding and high diffusion barrier to the template, as the template molecules were embedded inside the thick polymer network. Surface molecular imprinting method provides an alternative way to overcome these drawbacks, which attempts to build molecular recognition systems on the supporting materials surface [9–14]. The advantages of surface molecular imprinting include improving mass transfer, increasing affinity binding and decreasing high diffusion barrier of the template by fixing MIP on a support substrate. Up to now, various materials, such as magnetic nanoparticles [15–21], silica particles [22–24], nanowires/nanotubes [25–28], quantum dots (QDs) [29,30] and polystyrene nanoparticles [31] were chosen to be solid supporting materials for surface molecular printing. Among these materials, multi-walled carbon nanotubes (MWNTs), possessing extraordinarily large specific surface area as well as good mechanical properties, have been proven to be an available support material in surface imprinting process [32–39]. A special tip for fabricating a MIP on the surface of MWNTs was reported by several groups [34–39], in which vinyl groups were introduced onto the MWCNTs surface via covalent or non-covalent method to direct the selective polymerization of functional monomers and cross-linkers in the presence of the template. Another report introduced amine group on the surface of carbon nanotubes to fabricate an oleanonic-imprinted layer on the carbon nanotubes (CNTs) [32]. However, the introduction of double bond or amine group to the surface of carbon

* Corresponding author at: The Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi, Jiangsu 214122, China. Tel./fax: +86 510 8591 7763.

** Corresponding author.

E-mail addresses: jingluo19801007@126.com (J. Luo), lxy@jiangnan.edu.cn (X. Liu).

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nanotubes often involves harsh conditions and/or multiple reaction steps. In addition, the polymerization was mostly carried out in organic solvent at high temperatures, which is quite a disadvantage for imprinting biological molecules. Therefore, new approaches for the molecular imprinting on the surface of MWNTs are still highly desired.

On the other hand, while MIPs have been successfully developed against a wide range of small molecules, the imprinting of biomacromolecules, such as proteins, remains a challenge. The major difficulties associated with the imprinting of biomacromolecules include (i) the insolubility of proteins in commonly utilized imprinting solvents, (ii) the degradation of proteins under polymerization conditions such as high temperature, (iii) the large molecular size and structural complexity which restrict their mobility [8]. Surface imprinting is quite attractive for imprinting protein because the imprinted sites are close to or at the surface of MIP avoiding the protein entrapment in the polymer matrix and so enable the elution and rebinding of the target protein easily. However, as far as we are aware, no one has reported the imprinting of protein on the surface of MWNTs.

In recent years, the self-polymerization of dopamine on a wide variety of materials in aqueous solution to form polydopamine (PDA) coatings has been reported [40,41]. The resulted PDA film has a crosslinked structure to generate stable three-dimensional recognition sites. The thickness of the PDA film, which decides the depth of the imprinted cavities, is in nanoscale range and could be adjusted by changing the polymerization time. In addition, its multifunctional groups (amino and catechol groups), hydrophilicity and biocompatibility make it appropriate for imprinting bimacromolecules [42]. Several successful examples of employing PDA in molecular imprinting have been published [43–47].

Considering the advantages of MWNTs and PDA, the key idea of this paper is to develop a facile approach to imprint protein on the surface of MWNTs using dopamine as monomer. Just by mixing MWNTs, dopamine, template protein in weak alkaline aqueous solution, a thin adherent polydopamine (PDA) film imprinted with protein was spontaneously obtained on the surface of MWNTs to produce the imprinted nanomaterial (MWNTs@MIPs). This one-pot procedure avoids the surface-modification of MWNTs, representing a rapid, efficient and green approach to fabricate protein imprinted nanomaterials. The adsorption dynamics, special adsorption, and selective recognition of the MWNTs@MIPs were investigated. The results show that MWNTs@MIPs possess high rebinding capacity, specific recognition ability and good recycle performance towards template protein (papain) in aqueous media.

2. Experimental

2.1. Reagents and apparatus

MWNTs were purchased from Chengdu Organic Chemicals Co. Ltd. of Chinese Academy of Sciences. Dopamine hydrochloride (DA), papain (Pap), bovine serum albumin (BSA), lysozyme (Lyz), egg albumin and horseradish (HRP) were purchased from the Shanghai Alladin Chemical Reagent Company. All other chemicals were of analytical grade and used as received without further purification except for special statement. Doubly distilled water was used throughout the work.

UV–vis spectra were recorded on a TU-1901 spectro-photometer (Beijing Purkinje General Instrument Co., Ltd.). Raman was recorded with a Renishaw in via Raman Microscope operating at 514 nm with a charge-coupled device detector. XPS measurement was made on a VG ESCALAB MkII spectrometer with a Mg–Ka X-ray source (1253.6 eV photos). The X-ray source was operated at 14 kV and 20 mA. The morphologies of samples were determined using scanning electron microscopy (SEM) (Hitachi S-3700 N, Tokyo, Japan). TEM measurements were carried on a JEOL JEM-2100 microscope operating at 200 KV. Thermal gravimetric analysis (TGA) was conducted on a SDT 2960 instrument from room temperature to 850 °C with a heating rate of 20 °C min^{-1} in the nitrogen flow (10 mL min^{-1}). Gel electrophoresis for protein separation was carried out by regular sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 12% running gel and 5% stacking gel according to the manual introduction (Bio-Rad, Hercules, CA, USA). Proteins were stained with Coomasie Brilliant Blue R-250.

2.2. Preparation of MWNTs@MIPs

In a typical MWNTs@MIPs synthesis, MWNTs (20 mg) was dispersed in 20 mL Tris buffer (pH 8.0) by ultrasonication. 5 mg of papain and 16 mg of DA were then added. The mixture was stirred for 8 h at room temperature. After reaction, the product was washed with water to remove the unreacted monomer, then washed with a mixture of acetic acid (0.6 mol/L) and methanol (the ratio between acetic acid and methanol is 1:4, v/v) to extract the template protein and re-washed thoroughly with distilled water. For comparison, non-imprinted materials (MWNTs@NIPs) were prepared and treated under the same conditions but without the addition of the template protein.

2.3. Adsorption experiment of MWNTs@MIPs and MWNTs@NIPs

All the binding experiments were carried out in glass vials by using a batch technique. Before binding experiments, a calibration curve was obtained from the UV–vis spectra of the papain solutions with different concentrations. MWNTs@MIPs (10 mg) or MWNTs@NIPs (10 mg) was suspended in 10 mL papain solutions of different concentrations. The sample was incubated on a rocking table for 40 min at room temperature, then the mixture was centrifuged and the supernatant solution was collected. The concentration of free papain in the supernatant was measured by UV–vis at 276 nm. The papain bound was expressed as the difference between the total mass of papain loaded and mass of papain in solution after binding. The adsorption dynamics of the MWNTs@MIPs was performed by analyzing the free papain concentration in the supernatant at different time intervals.

2.4. Selectivity experiments

In the selectivity experiments, bovine serum albumin (BSA), lysozyme (Lyz), egg albumin were chosen as the reference substrates to investigate the selectivity to the template protein. For the separative adsorption experiments, the different protein solutions (each with a concentration of 0.3 mg mL^{-1}) were applied to bind with the imprinted and non-imprinted materials respectively. The resulting concentrations of BSA, Lyz, and albumin in the supernatant were measured by the UV–vis separatively. For the mixed adsorption experiments, Lyz and HRP were chosen as the competitive protein. The adsorption was performed within a protein mixture (containing papain, Lyz and HRP). Ten microliter of the mixed solution, before and after the adsorption, was extracted for SDS-PAGE analysis.

3. Results and discussion

3.1. Preparation and characterization of papain imprinted MWNTs@MIPs

Fig. 1 depicts the schematic representation of MWNTs@MIPs preparation. A simple mixture of MWNTs and dopamine in a weak