Journal of Colloid and Interface Science 440 (2015) 53–59

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Enhanced surface imprinting of lysozyme over a new kind of magnetic chitosan submicrospheres

Hao Guo, Dongying Yuan, Guoqi Fu *

Key Laboratory of Functional Polymer Materials of Ministry of Education, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Institute of Polymer Chemistry, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 11 September 2014
Accepted 23 October 2014
Available online 4 November 2014

Keywords:
Surface imprinting
Protein imprinting
Molecular imprinting
Magnetic chitosan microspheres
Lysozyme recognition

ABSTRACT

Surface protein imprinting over nano- or micron-sized substrates is an effective approach for improving the biomacromolecule mass transfer and rebinding capacity. For achieving high recognition performance, it is necessary to introduce certain functional groups onto the surface of the support materials which can interact with the template protein. Herein, we report a surface protein imprinting approach using a new kind of core–shell magnetic chitosan submicrospheres as the supports. The surface of these magnetic chitosan particles is tethered with uncross-linked chitosan chains, hence bearing plenty of amino and hydroxyl groups, where a large amount of functional ligands can be readily coupled for capturing of the protein template. With lysozyme as a model print protein, the magnetic supports were functionalized with maleic acid and then coated with imprinted polymer layers. The resulting imprinted microspheres show significantly selective rebinding for lysozyme. In particular, they exhibit a specific rebinding capacity about three times higher than achieved with our previous lysozyme-imprinted particles synthesized in similar way but with maleic acid modified silica nanoparticles as the supports. This can be attributed to the much higher template binding capacity to the modified magnetic chitosan submicrospheres. Also, the resultant imprinted particles can be easily collected by a magnet. Therefore, such kind of chitosan submicrospheres may be a versatile carrier for constructing high-capacity and magnetically recyclable surface protein-imprinted particles.

1. Introduction

Molecular imprinting is a facile technique for the synthesis of tailor-made artificial recognition materials by copolymerizing suitable functional monomers in the presence of desired template molecules. After the removal of the template, the resulting molecularly imprinted polymers (MIPs) are complementary in shape, size, and functionality with respect to the template and can rebind the target molecules with high selectivity and affinity. Compared with natural recognition materials such as antibodies, MIPs exhibit advantages such as stability, specific recognition and ease of mass preparation and hence have found applications in wide areas including separation, sensors, catalysis, and drug delivery [1,2]. To date, the molecular imprinting technology against small molecules has been well-established. However, the successful imprinting of proteins and other biomacromolecules is still facing a great challenge. The major problems have been identified, including the restricted mass transfer across the cross-linked polymer matrix due to the large molecular sizes, significantly reduced template–monomer interaction in aqueous media where proteins prefer to exist, flexible and complex structure which is susceptible to harsh synthesis conditions [3–6]. Aiming at overcoming the problem associated with the mass transfer difficulty, a variety of strategies have been exploited, such as surface imprinting, adopting nano-sized physical forms (e.g. nanoparticles and nanofilms), and epitope imprinting with a fragment of the original macromolecular target as a template. Particularly, nano-sized protein-imprinted materials have received great attention due to their high surface-to-volume ratio which is expected to facilitate template removal, improve accessibility of the generated recognition sites, and elevate both binding capacity and kinetics [7,8]. Therefore, surface protein imprinting over preformed nanomaterials, based on combination of the surface imprinting strategy with a variety of nanotechniques, has so far proved to be a promising approach, and Lv et al. [9] have extensively reviewed these studies.

With surface imprinting of a protein over support materials, not only the macromolecule transfer problem can be alleviated, but also the imprinting performance can be improved by introducing surface functional ligands for interaction with the template protein

* Corresponding author.
E-mail address: gqfu@nankai.edu.cn (G. Fu).

http://dx.doi.org/10.1016/j.jcis.2014.10.059
0021-9797/

before formation of the imprinted layer. Template molecules can be immobilized on the surface via either reversible covalent bonding (like imine bond and phenylboronic acid ester), or affinity interactions such as those based on an enzyme and its inhibitor [10–14]. Also, the template proteins can be physically adsorbed on the surface of the supports modified with suitable functional groups [15–19]. The latter strategy based on physical uptake has been more widely employed due to much easier surface functionalization and subsequent template removal in contrast with the covalent template immobilization approach. For example, silica (nano)particles modified with amino, carboxyl or metal chelating groups via silicane coupling agents have been often employed for surface protein imprinting. However, the rebinding capacity of the resulting core–shell protein-imprinted particles is still relatively low, probably due to the amount of functional groups coupled to the support particles' surface is limited and hence leading to low template binding prior to forming the imprinted shell. To overcome such problem, Ulbricht et al. [20,21] and Lin et al. [11] recently proposed an alternative route for surface modification of the support materials, that is, surface grafting of polymer chains which bear a large amount of functional groups. As-modified support materials can capture more template protein molecules than modified directly on the surface. Ulbricht and coworkers [20,21] reported a two-step surface grafting method for imprinting of lysozyme or immunoglobulin G on the surface of porous membrane which is grafted with poly(methacrylic acid) (PMAA) brushes via surface initiated ATRP of poly(tert-butyl methacrylate) and subsequent hydrolysis. In the second step for grafting of surface imprinted polymer layer, the plentiful carboxyl groups on the PMAA chains can bind the template proteins via electrostatic attraction or hydrogen bonding. In this method, however, the process for grafting of PMAA chains is rather tedious.

Recently, we reported a facile aqueous one-pot method for synthesis of core–shell structured magnetic chitosan (CS) submicrospheres [22]. These composite microspheres are characterized by their surface being tethered with uncross-linked CS chains. By virtue of plentiful reactive $-NH_2$ and –OH groups in the CS chains, these magnetic CS submicrospheres after suitable modification may be a versatile support for grafting of protein-imprinted shells with enhanced recognition performance. For proof of this hypothesis, herein such magnetic CS particles were modified with maleic anhydride to introduce both polymerizable double bonds and carboxylic groups which can interact with positively charged proteins. With basic lysozyme as a model protein template, protein-imprinted layer was then formed over the modified CS particles using a previously reported approach [15,23,24]. As-prepared lysozyme-imprinted microspheres showed greatly increased specific rebinding capacity in comparison with our previously reported imprinted particles synthesized in a similar way but with maleic acid modified silica nanoparticles as the supports. Also, the resultant imprinted particles were highly magnetic and could be readily recycled by an external magnetic field.

2. Experimental

2.1. Materials

CS powder was purchased from Yuhuan Biochemical Co., Ltd. (Zhejiang, China), with a viscosity-average molecular weight of about 40,000 and deacetylation degree of about 90%. Acrylic acid (AA) and methacrylic acid (MAA) (Tianjin Chemical Reagents Co., Tianjin) were purified by vacuum distillation, respectively. (Dimethylamino)ethyl methacrylate (DMAEMA, Alfa Aesar) was purified by passing through a basic alumina column. Potassium persulfate (KPS, Sinopharm Chemical Reagent Co. Ltd., Shanghai, AR) and Ammonium persulfate (APS, Tianjin Chemical Reagents Co., Tianjin, AR) were recrystallized in water, respectively. Ethylene glycol (Guangfu Chemical Reagents Co. Tianjin, AR), sodium acetate (Guangfu Chemical Reagents Co. Tianjin, AR), trisodium citrate dehydrate (Guangfu Chemical Reagents Co. Tianjin, AR), $FeCl_3$ (Alfa Aesar, 99.9%), acrylamide (AAm, Sangon, Shanghai, electrophoresis grade), N,N-methylenebisacrylamide (MBA, Sangon, Shanghai, electrophoresis grade), Lysozyme (Lyz, Dingguo Biotech Co., Beijing), bovine serum albumin (BSA, Dingguo Biotech Co., Ltd., Beijing), Cytochrome c (Cyt c, Sangon, Shanghai), Bovine hemoglobin (Hb, Sigma), ribonuclease A (RNase A, Sigma), and N,N,N′,N′-tetramethylethylenediamine (TEMED, Aladdin, China), and all the other chemicals were used as received unless otherwise stated.

2.2. Characterization

Transmission electronic microscopy (TEM) images were taken using a JEOL TEM operated at 100 kV. Samples were dispersed in ethanol, cast onto a carbon-coated copper grids, and then dried under vacuum. Hydrodynamic diameters (D_h) and zeta potentials of the particles were measured by dynamic light scattering (DLS) with a Malvern ZEN3600 Zetasizer Nano instrument using a He–Ne laser at a wavelength of 632.8 nm. Fourier-transformation infrared (FT-IR) spectra were determined on a Bio-Rad FTS 135 FTIR spectrometer over KBr pellets. The magnetic properties were studied with a vibrate sample magnetometer (VSM, 9600, BOJ Electronics) at room temperature. Thermogravimetric analysis (TGA) was performed for the particle samples (~10 mg) using a NETZSCH TG 209 thermogravimetric analyzer under a nitrogen atmosphere with a heating rate of 10 °C/min up to 900 °C.

2.3. Synthesis and modification of magnetic chitosan submicrospheres

Firstly, magnetite colloid nanocrystal clusters (MCNCs) bearing carboxylic groups were prepared according to the method described previously [25]. Briefly, a mixture consisting of $FeCl_3$ (3.9 g), trisodium citrate dehydrate (1.8 g), and sodium acetate (7.2 g) thoroughly dissolved in ethylene glycol (120 mL) was sealed in a Teflon-lined stainless-steel autoclave, heated to 200 °C and maintained for 10 h. The black particles were thoroughly washed sequentially with ethanol and deionized water and finally dried under vacuum.

The magnetic chitosan submicrospheres were then synthesized by copolymerization of AA and MBA in CS solution mixed with the aforementioned MCNCs according to our previous work with some modification [22]. In brief, a suspension containing AA (110 mg), MBA (11 mg), CS (250 mg), and MCNCs (200 mg) mixed in 80 mL of deionized water was sonicated under mechanical agitation for 30 min for sufficient preassembly and then heated to 70 °C together with continued stirring and driving out air using nitrogen stream. After adding 44 mg of KPS dissolved in 2 mL of deionized water, the polymerization was carried out for 2 h. The resulting magnetic polymer particles (hereafter named as Fe_3O_4@PAA/CS) were enriched with the help of a magnet, washed sequentially with 1% HAc solution and deionized water, and finally freeze-dried.

For reaction with maleic anhydride, the dry Fe_3O_4@PAA/CS particles (2.0 g) were mixed with N,N-dimethylformamide (100 mL) containing maleic anhydride (4 g) and 4-dimethylaminopyridine (400 mg), and the dispersion was stirred at 60 °C overnight. Finally, the product (denoted as Fe_3O_4@PAA/CS–COOH) was collected by magnetic separation, washed extensively with ethanol and water, and finally freeze-dried.

2.4. Preparation of Lyz-imprinted particles

Synthesis of surface Lyz-imprinted particles was carried out according to our work reported previously [15,23,24]. Typically,