Biosensors and Bioelectronics 53 (2014) 5–11

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Synthesis of surface molecularly imprinted nanoparticles for recognition of lysozyme using a metal coordination monomer

Huachang Chen, Juan Kong, Dongying Yuan, Guoqi Fu *

Key Laboratory of Functional Polymer Materials of Ministry of Education, Institute of Polymer Chemistry, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form
14 September 2013
Accepted 17 September 2013
Available online 25 September 2013

Keywords:
Molecular imprinting
Protein imprinting
Surface imprinting
Nanostructures
Lysozyme
Metal-coordination

ABSTRACT

Molecularly imprinted polymers against proteins are regarded as promising substitutes for natural antibodies, but have been frustrated with the problems including reduced interaction between functional monomers and protein template in the aqueous media required during their synthesis and restricted mass transfer across the resulting crosslinked polymer matrixes. For addressing these issues, herein we proposed a strategy for imprinting of a protein on the surface of nanoparticles using a metal chelating monomer. With lysozyme as a model protein template and Cu^{2+} chelating N-(4-vinyl)-benzyl iminodiacetic acid as the coordination monomer along with other monomers, protein imprinted polymer nanoshells were formed over vinyl-modified silica nanoparticles via surface polymerization in high-dilution monomer solution. The feed concentration of the crosslinking monomer was optimized toward achieving the best imprinting effect. Compared with the related imprinted materials reported previously, the resultant core–shell imprinted particles showed greatly faster binding kinetics, elevated rebinding capacity and selectivity. More importantly, noticeably high binding affinity was achieved with an estimated dissociation constant of 4.1×10^{-8} M which is comparable to that of conventional antibodies

1. Introduction

Molecular imprinting is a promising technique for artificial formation of affinity recognition sites in a synthetical matrix. It involves polymerization of functional and crosslinking monomers in the presence of a template molecule and subsequent removal of the template form the resulting polymers. The so-called molecularly imprinted polymers (MIPs) thus obtained are complementary in shape, size, and functionality with respect to the template and facilitate selective rebinding of the template molecules. Compared with natural recognition materials like antibodies, MIPs offer advantages like stability, specific recognition and ease of mass preparation, and hence have found applications in wide areas including separation, sensors and catalysis (Bergmann and Peppas, 2008; Haupt and Mosbach, 2000; Marty and Mauzac, 2005). Besides the well established imprinting of small molecules, in recent years the MIPs against proteins and other biologically relevant targets have attracted increasing research interest, since they are regarded as the most potential alternatives for the expensive and labile antibodies, which are now widely used in the areas such as biosensors, bioseparation and medical diagnostics. However, imprinting of proteins still faces a great challenge toward realizing what are expected. One problem lies in the restricted mass transfer across the crosslinked polymer matrix due to the large molecular sizes, which restricts the ease of template removal as well as rebinding. Other problems include significantly reduced non-covalent template–monomer interaction in aqueous media where proteins prefer to exist, complex and flexible structures of proteins, all of which are disadvantageous to the creation of high-quality imprints for selective or even specific recognition (Bossi et al., 2007; Kryscio and Peppas, 2012; Verheyen et al., 2011; Whitcombe et al., 2011).

Principally aiming at overcoming or alleviating the problem associated with the mass transfer difficulty, a variety of strategies for protein imprinting have been exploited, such as surface imprinting, epitope approach, utilization of moderately cross-linked hydrogels, and adopting nanosized physical forms such as nanoparticles and nanowires (Bossi et al., 2007; Kryscio and Peppas, 2012; Verheyen et al., 2011; Whitcombe et al., 2011). The nanosized protein-imprinted materials with extremely high surface-to-volume ratio are expected to facilitate template removal, improve accessibility of the generated recognition sites, and elevate both binding capacity and kinetics (Gao et al., 2007; Li et al., 2006). Also, the protein-imprinted nanostructures are particularly suitable for in vivo therapy (Cutivet et al., 2009; Hoshino et al., 2010) and the construction of biosensors (Cai et al., 2010; Dechtrirat et al., 2012; Moreira et al., 2011a; Sener et al., 2011). Till now, a lot of nanotechniques have been explored for the imprinting of protein molecules. Among these, the

* Corresponding author. Tel.: +86 22 23501443.
E-mail address: gqfu@nankai.edu.cn (G. Fu).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.037

approaches based on attaching imprinting layers on the surface of the preformed nanosized support materials have recently aroused much more research interest due to their additional features in comparison with the homogeneously structured protein-imprinted nanomaterials, such as further ease of template diffusion, ready control over the size or form of the final MIPs, and facile integration of other functions with molecular imprinting (Chen et al., 2012, 2013; Fu et al., 2011; Gai et al., 2010; Gao et al., 2011; He et al., 2010; Inoue et al., 2011; Jing et al., 2010; Kan et al., 2010; Lee et al., 2010; L. Li et al., 2009; S.H. Li et al., 2009, ; Y.X. Li et al., 2013a, 2013b; Liu et al., 2011; Moreira et al., 2011b. Turner et al., 2007; Wang et al., 2008; Xia et al., 2013; M Zhang et al., 2012a W. Zhang et al., 2012a, 2012b). Lv et al. (in press) have extensively reviewed the studies on the surface imprinting of protein over nanomaterials.

Compared with the aforementioned studies addressing the biomacromolecule transfer limitation, relatively fewer efforts have been made for improving the quality of protein imprints in terms of rebinding selectivity and affinity. With regard to the widely employed surface imprinting strategy, reversible covalent immobilization (Gao et al., 2011; Inoue et al., 2011; Y.X. Li et al., 2013; Moreira et al., 2011a, 2011b; Shiomi et al., 2005) or non-covalent adsorption (Fu et al., 2011; Liu et al., 2011; M. Zhang et al., 2012) of template proteins on the support materials prior to the surface imprinting process has proved to be effective approaches for enhancement of the imprinting effect. Another important approach involves the design of protein-imprinted polymers based on using monomers with moieties already known to interact strongly with the target molecule, for example, taking advantage of boronic acid–diol (Glad et al., 1985; L. Li et al., 2013), enzyme–inhibitor (Cutivet et al., 2009; Vaidya et al., 2001), or metal coordination (Bereli et al., 2008; L. Li et al., 2009; S.H. Li et al., 2009; Kempe et al., 1995; Qin et al., 2009) interactions, and introduction of the specially designed macromonomers, i.e. assistant recognition polymer chains which were named by the authors (Guo et al., 2006). Among these interactions, the metal coordination may be more viable, since lots of proteins have exposed histidine (His) residues, which can form rather stable coordination complex with a metal ion-chelating monomer. Furthermore, such metal coordination interaction is nearly hindered by the presence of aqueous media which are required for protein imprinting. Also, after producing the imprinted polymers via polymerization, the strong coordination interaction can be readily destroyed via removal of the metal ions (e.g. by washing with aqueous EDTA solution), hence facilitating template removal. Several research groups have reported on the synthesis of protein-imprinted hydrogels or cryogels using metal chelate monomers based on the bulk polymerization method (Bereli et al., 2008; L. Li et al., 2009; S.H. Li et al., 2009; Qin et al., 2009). As-prepared imprinted materials show rather good imprinting performance in terms of both rebinding capacity and selectivity; however, the binding kinetics is greatly hampered due to the intrinsic drawback of the bulk polymerization approach employed. Also, it is worth noting that the resultant imprinted gels do not exhibit prominent binding affinity as expected.

In this work, we demonstrate surface protein imprinting over silica nanoparticles with lysozyme as a model biomacromolecular template and using a metal coordination monomer *N*-(4-vinyl)-benzyl iminodiacetic acid (VBIDA) in the prepolymerization recipes. The formation of the imprinted polymer nanoshells is based on our previously reported strategy, i.e. graft copolymerization on the surface of vinyl-modified nanoparticles in highly dilute monomer solution (Chen et al., 2013; Fu et al., 2011; He et al., 2010). The feed crosslinker contents were optimized toward achieving the best imprinting effect. The resulting core–shell imprinted nanoparticles showed significant improvement in not only rebinding kinetics but also rebinding affinity, compared with the previously reported hydrogels based imprinted polymers synthesized with the similar monomer composition.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAAm, Acros) was purified by recrystallization from 60/40 hexane/toluene mixture. Ammonium persulfate (APS, Tianjin Chemical Reagents Co., Tianjin, AR) was recrystallized from water. Tetraethylorthosilicate (TEOS, Tianjin Chemical Reagents Co., Tianjin) was distillated before use. 3-Methacryloxypropyl trimethoxysilane (MPS, Diamond Advanced Materials Inc., Hubei, China), 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. VBIDA was synthesized according to a previously published procedure (Morris et al., 1959).

2.2. Characterization

Transmission electronic microscopy (TEM) images were taken on a JEOL TEM operated at 100 kV. Samples were dispersed in ethanol at an appropriate concentration, cast onto a carbon coated copper grids and then dried under vacuum. Thermogravimetric analysis (TGA) was carried out using a thermogravimetric analyzer (TG 209, Netzsch) under nitrogen atmosphere with a heating rate of 10 °C min^{-1} up to 900 °C. Elemental analysis was performed with an elemental analyzer (Elementar Vario EL, Elementar).

2.3. Synthesis of Lyz-imprinted nanoparticles

Silica nanoparticles were synthesized by the Stöber method and subsequently modified with MPS as described in our previous work (He et al., 2010). Typical Lyz-imprinted nanoparticles were prepared as follows. VBIDA (11.2 mmol) was dissolved in 2 mL of distillated water assisted by adding a small amount of NaOH solution (0.1 mM, ~50 μL), and then mixed with $CuSO_4 \cdot 5\,H_2O$ (11.2 mmol) for 1 h followed by mixing with Lyz (30 mg) for another 1 h. This solution was mixed with 11.5 mL of phosphate buffer (pH 7.0, 10 mM) containing NIPAAM (40.5 mg), AAm (1.9 mg), and MBA (30 mg), and the suspension of the MPS modified silica nanoparticles (45 mg) dispersed in 1.5 mL of ethanol. After 1 h shaking for preassembly, the mixture was degassed under vacuum for 10 min and purged with nitrogen stream for another 10 min. By injecting 45 μL of APS solution (10%, w/v) and 23 μL of TEMED solution (5%, v/v) to the dispersion, polymerization was initiated and continued under violent starring at 25 °C for 24 h. The resultant particles were collected by centrifugation, washed sequentially with distillated water to remove the adsorbed oligomers and unreacted monomers, with EDTA solution (0.1 M) to desorp Cu^{2+}, with 0.5 M NaCl solution to remove embedded template until no Lyz in the supernatant was detected using a UV/Vis spectrophotometer at 280 nm, and with distillated water to remove remaining NaCl. No significant conformation change was noted for the desorped Lyz as compared with the native Lyz (see supporting information, Fig. S1). The particles were then loaded with Cu^{2+} by incubation in $CuSO_4$ solution (0.1 M) for 30 min, washed extensively with distillated