Analytica Chimica Acta 909 (2016) 60–66

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Poly(amino acid)-based thermoresponsive molecularly imprinted magnetic nanoparticles for specific recognition and release of lysozyme

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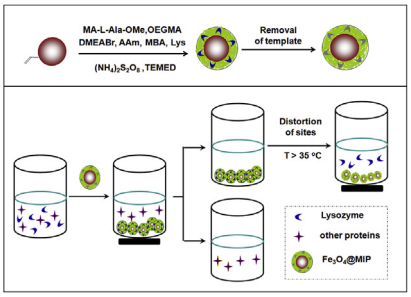
HIGHLIGHTS

- Poly(MA-L-Ala-OMe) based MIPs for enrichment and release of Lys have been prepared.
- Capturing and releasing of Lys could be facilitated by using the thermo-responsive MIPs.
- The MIPs have exhibited a great potential in enriching Lys in practical application.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 October 2015
Received in revised form
14 December 2015
Accepted 2 January 2016
Available online 11 January 2016

Keywords:
Poly(amino acid)
Thermoresponsive
Molecularly imprinted polymers (MIPs)
Surface imprinting magnetic particles
Lysozyme

ABSTRACT

In this study, poly(amino acid)-based thermoresponsive molecularly imprinted magnetic nanoparticles for recognition and release of lysozyme was prepared via surface imprinting method. For constructing the molecularly imprinted polymer (MIP) layer, amino acid-based thermoresponsive monomer (N-methacryloyl-L-alanine methyl ester, MA-L-Ala-OMe) was mainly selected for the functional monomer along with N,N′-methylenebis(acrylamide) as the crosslinker. The resultant magnetic MIP nanoparticles were characterized in detail. Meanwhile, the dynamic light scattering studies and swelling ratios measurements were carried out for demonstrating the thermoresponsive property of the imprinted nanoparticles. The prepared magnetic MIP nanoparticles showed good adsorption capacity and selective recognition properties to lysozyme. Moreover, the fast adsorption process could reach equilibrium within 15 min. Importantly, the capture and release of lysozyme could be easily realized simply by altering the temperature of aqueous solution. Furthermore, the prepared imprinted nanoparticles were applied to

Abbreviations: MIPs, molecularly imprinted polymers; MA-L-Ala-OMe, N-methacryloyl-L-alanine methyl ester; PNIPAAm, poly(N-isopropylacrylamide); LCST, tunable phase transition temperature; DMEABr, N-ethyl-2-(methacryloyloxy)-N,N-dimethylethananminium bromide; OEGMA, poly(ethylene glycol) methyl ethermethacrylate; MBA, N,N′-methylenebis(acrylamide); Lys, lysozyme; Pep, pepsin; Mb, myoglobin; BSA, bovine serum albumin; L-Ala-OMe·HCl, L-alanine methyl ester hydrochloride; APS, ammonium persulfate; TEMED, N,N,N′,N′-Tetramethylethylenediamine; DMAEMA, dimethylaminoethyl methacrylate; TFA, trifluoroacetic acid; MPS, γ-methacryloxypropyltrimethoxysilane; FT-IR, Fourier transform infrared; DLS, dynamic light scattering; Fe_3O_4@MIP/NIP, Fe_3O_4@MIP/NIP nanoparticles; Q, adsorption capacity; α, imprinting factor.

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http://dx.doi.org/10.1016/j.aca.2016.01.005

separate lysozyme from the real egg white samples. The results proved that the thermoresponsive MIPs based on MA-L-Ala-OMe have great potential for selectively enriching target proteins in real samples.

1. Introduction

Molecular imprinting is a versatile technique to produce polymers-based receptors, which can specially recognize and rebind template molecule [1]. Compared with natural receptors (such as enzymes), molecularly imprinted polymers (MIPs) have many advantages of low cost, easily available, high chemical and excellently mechanical stability. Therefore, MIPs have attracted more attention over the past few decades in many research areas, e.g. separation [2–4], solid phase extraction [5–7] and bio-mimetic sensors [8–12]. In general, according to the different sizes of template molecules, MIP materials can be divided into small molecules and large molecules-based MIPs. To date, most of successful MIPs have been based on small target molecules [13–16]. However, the imprinting of biomacromolecules, especially target proteins, is still a more difficult task: on one hand, the low capturing and releasing rate results from the restricted mass transfer and complex structure. On the other hand, large size and flexible molecular structure of macromolecules can lead to the difficulties in removing template molecules [3]. In despite of these difficulties, the development of MIPs for protein-MIPs is significantly important due to their great potential in the biorelated-applications, such as diagnosis, therapy, and so on. Therefore, many attempts for isolation and purification of interesting proteins have been explored in recent years [17–20].

Up to date, different methods have been employed to synthesize protein-MIPs, such as surface imprinting, epitope imprinting and bulk imprinting. Among them, surface imprinting could overcome the restricted mass transfer due to the recognition sites locating at the surface of polymers or near the surface, resulting in high adsorption rate. Thus, in the last decades, surface imprinting has been widely used to prepare protein-MIPs [21,22]. Meanwhile, magnetic nanoparticles are one of the most extensively used substrates for preparation of surface imprinting materials which have large surface areas, good biocompatibility and high chemical stability. More importantly, the synthesized molecularly imprinted magnetic nanoparticles can be fast separated by an external magnetic field without complicated isolation procedure (e.g. centrifugation) [23,24].

Although the previous reports [23,24] have offered the helpful hint to overcome the limitations for constructing protein-MIPs by taking the surface imprinting strategy, another key factor that limits the further application of protein-MIPs is difficulty in removal of template molecules. In traditional method, template proteins could be washed from the imprinted caves by the relatively harsh conditions [1], for example, utilizing acetic acid-sodium dodecyl sulfate sodium solution as the washing buffer [22]. Meanwhile, the severe conditions of elution were often time-consuming for removing the template proteins. Additionally, the extreme conditions for removing template molecules may result in the unfavorable influence on the adsorption performance. To resolve this problem and enlarge the application range of MIPs, endowing MIP with stimuli responsive properties might effectively overcome above limitations. Because the capture and release of target molecules could be easily achieved by altering the external conditions, therefore, the responsive MIPs has drawn considerable attentions [25–28]. Among these responsive materials, thermoresponsive polymers have been widely applied for superiority of the easily controlled temperature. Poly(N-isopropylacrylamide) (PNIPAAm) is the main themoresponsive polymer in preparation of thermoresponsive protein-MIPs [29–32]. However, kinds of available thermoresponsive polymers is still limited up to date. Therefore, the development of novel thermoresponsive polymers for preparing protein-MIPs is highly desirable and meaningful.

The newly emerging poly(amino acids)-based polymers possess relatively good thermoresponsive properties [33] and might be considered as the idealistic candidates. Furthermore, their good biocompatibility and tunable phase transition temperature (LCST) could lead to successful application in the thermoresponsive materials. Moreover, the special characteristics of poly(amino acids), together with their facile preparation procedure, make them have outstanding potential for constructing thermoresponsive protein-MIPs. However, to our best knowledge, such an attempt has not been explored.

Herein, we described the first study for preparing poly(amino acid)-based thermoresponsive magnetic MIP nanoparticles via surface imprinting method. In the MIP layer, N-methacryloyl-L-alanine methyl ester (MA-L-Ala-OMe), an amino acid derivative, was selected to be the functional and thermoresponsive monomer. N-ethyl-2-(methacryloyloxy)-N,N-dimethylethananminium bromide (DMEABr) and poly(ethylene glycol) methyl ether-methacrylate (OEGMA) were selected as the assistant monomers with N,N′- methylenebis(acrylamide) (MBA) as the crosslinker. The thermoresponsive imprinted polymer layer was anchored onto the surface of the magnetic nanoparticles. The resultant MIP nanoparticles were comprehensively characterized and their thermoresponsive behavior was studied. Meanwhile, their binding properties to lysozyme (Lys) were investigated. Interestingly, the resultant MIPs were able to autonomously capture and release the template Lys simply by controlling the external temperature. Moreover, the prepared MIP nanoparticles could achieve quick adsorption to Lys at a high speed and specifically separate Lys from biological sample without tedious pretreatment procedure.

2. Experimental section

2.1. Reagents and materials

Lys, pepsin (Pep), myoglobin (Mb) and bovine serum albumin (BSA) in this study were obtained from Xin Jing Ke Biotechnology (Beijing, China). L-alanine methyl ester hydrochloride (L-Ala-OMe·HCl, 98%), methacryloyl chloride (95%), 2-(dimethylamino) ethyl methacrylate (99%), were provided by Aladdin Industrial Corporation (Shanghai, China). Ammonium persulfate (APS, ≥99%), acrylamide (AAm, ≥98%), N,N′-methylenebis(acrylamide) (MBA, ≥99%), $FeCl_3 \cdot 6H_2O$ (≥99%), and $FeCl_2 \cdot 4H_2O$ (≥99%) were purchased from Beijing Chemical Plant.

N,N,N′,N′-Tetramethylethylenediamine (TEMED, 98%) was purchased from Beijing Mairui Da Technology Co. Ltd. (Beijing, China). Dimethylaminoethyl methacrylate (DMAEMA), trifluoroacetic acid (TFA), γ-methacryloxypropyltrimethoxysilane (MPS, 98%) were supplied by Beijing InnoChem Science and Technology Co. Ltd. (Beijing, China). HPLC grade acetonitrile was purchased from Beijing Shengshi Chuangqi Science and Technology Co. Ltd. (Beijing, China). All other reagents used were analytical grade.