Applied Surface Science 357 (2015) 1490–1498

Contents lists available at ScienceDirect

Applied Surface Science

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

Facile preparation of superparamagnetic surface-imprinted microspheres using amino acid as template for specific capture of thymopentin

Longxia Guo, Xiaoling Hu *, Ping Guan, Chunbao Du, Dan Wang, Dongmen Song, Xumian Gao, Renyuan Song

Key Laboratory of Applied Physics and Chemistry in Space of Ministry of Education, School of Science, Northwestern Polytechnical University, Xi'an 710072, China

ARTICLE INFO

Article history:
Received 4 May 2015
Received in revised form 2 October 2015
Accepted 6 October 2015

Keywords:
Surface imprinting
Fragment imprinting
Amino acid
Thymopentin
Magnetic microspheres

ABSTRACT

Novel superparamagnetic surface-imprinted microspheres (SIMs) with molecularly imprinted shell layer were controllably synthesized via fragment imprinting and surface imprinting technique. The SIMs-Arg and SIMs-Lys microspheres were prepared by using L-arginine (L-Arg) and L-lysine (L-Lys) as pseudo-template molecule for specific rebinding to thymopentin (TP5), respectively. The characterization results revealed that both SIMs-Arg and SIMs-Lys were successfully prepared and possessed a high magnetic sensitivity. The rebinding-isotherm analyses of SIMs-Arg and SIMs-Lys showed that the Langmuir isotherm model was well fitted to the equilibrium data, indicating that only one kind of rebinding site was present in SIMs-Arg and SIMs-Lys. Besides, the kinetic properties of SIMs-Arg and SIMs-Lys both were well described by the pseudo-second-order kinetics model, which indicated that a chemical process may be the rate-limiting step in the rebinding process. Moreover, the magnetic imprinted microspheres were found to have a higher specificity for TP5 than that for immunostimulating peptide human (IPH). What is more, SIMs-Arg and SIMs-Lys were successfully applied for TP5 determination in urine. According to the maximum adsorption capacity, the imprinting factor and real sample experiment, it was noted that SIMs-Arg had better specific adsorption property for TP5 than SIMs-Lys.

1. Introduction

Thymopentin (TP5, Fig. 1), a pentapeptide arginyl-lysyl-aspartyl-valyl-tyrosine, containing residues 32–36 of the thymic hormone thymopoietin, corresponds to an active site of thymopoietin and serves as a therapeutic substitute for thymopoietin [1]. TP5 has been clinically used in the prevention and treatment of autoimmune diseases, such as atopic dermatitis [2], Sézary's syndrome [3], and cancers [4]. Besides, TP5 not only acts as an immunomodulatory factor in cancer chemotherapy, but also is a potential chemotherapeutic agent in the human leukemia therapy [5]. What is more, the researchers have found that thymopentin could reduce "typical" inflammatory response, which may be promising as a disease-prevention service, thereby diminishing the risk of fatal consequences of multiple sclerosis in humans [6]. TP5 is currently isolated and purified primarily using two methods, reversed-phase chromatography [7] and ion-exchange chromatography [8]. However, these two methods have low efficiency and high operating cost. Therefore, a separation methodology with high selectivity for TP5 is urgently needed.

The technique of molecular imprinting is a powerful tool to prepare polymers with specific recognition, strong affinity and high selectivity for target molecules [9,10]. Molecularly imprinted polymers (MIPs) were synthesized through the copolymerization of one or more functional monomer-template complexes with a crosslinking agent [11–14]. Following the removal of the template molecules, the cavities were produced which were complementary to the templates in shape, size and functional group orientation. Because of their predetermined selectivity and recognition, MIPs have attracted considerable interest in some fields that are involved in clinical analysis, environmental monitoring and drug delivery [15–17]. Up to now, the template molecule was directly used during the process of preparation of MIPs, thus MIPs for low-molecular-weight templates are well established [18–20]. However, due to

* Corresponding author.
E-mail address: huxl054@163.com (X. Hu).

http://dx.doi.org/10.1016/j.apsusc.2015.10.042
0169-4332/

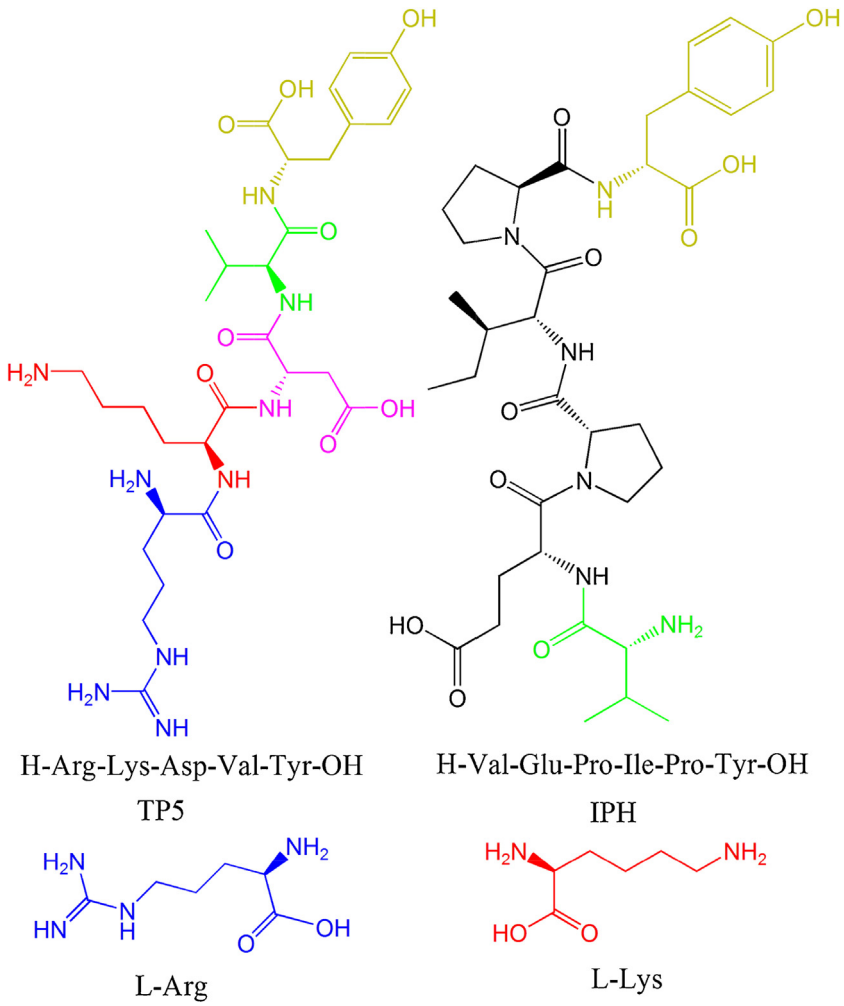

Fig. 1. Molecular structures of biomolecules were used in this study.

the harsh and complex environment of polymerization process and sensitive structural nature of biomolecule, choosing biomolecule as the template in the preparation of MIPs, such as peptides and proteins, limits success [21–23]. In addition to this, the imprinted cavities generating in the most biomolecule imprinting polymers were not only on the surface but also deeply inside the network structure, so the biomolecules could not be removed completely from the MIPs even after exhaustive repeated washing with a range of solvents [24]. To overcome the problem that exists in biomolecule imprinting, the fragment imprinting technique combining with surface imprinting, which utilized one segment of the target as the template to prepare MIPs and only generated recognition sites on the surface of MIPs would be a promising method. According to this method, MIPs could be synthesized using a pseudo-template to selectively capture and enrich target molecule in real samples, and the imprinted sites were only distributed on the surface of MIPs.

Compared with the traditional solid support substrate, the magnetic microspheres with their unique properties, such as higher surface area-to-volume ratio, superparamagnetism, high magnetic saturation and low toxicity have recently attracted increasing attention on synthesizing MIPs [25]. Because of the high magnetic susceptibility, MIPs rebinding target molecules can be readily collected and isolated from the complex matrix by an external magnetic field without additional centrifugation or filtration [26]. In recent years, Fe_3O_4 magnetic nanoparticles with simple preparation and low cost have been extensively used in MIPs [27].

In this work, we have prepared the novel superparamagnetic surface-imprinted microspheres (SIMs) to selectively recognize biological drug molecule TP5 by using the end group (L-arginine, L-Arg) and side chain (L-lysine, L-Lys) of TP5 as template from aqueous medium. For fragment imprinting, the end groups and side chains of analytes play a particularly important role in the whole recognition process [28–30]. As shown in Fig. 1, L-Arg, L-Lys, L-tyrosine (L-Tyr) and L-aspartic acid (L-Asp) are the end groups or side chains of TP5. Theoretically, they all should be selected as the template molecule respectively. However, L-Tyr is almost not soluble in aqueous medium, and L-Asp is also not suitable as template when considering methacrylic acid (MAA) as functional monomer [31]. KH-570 was firstly introduced to the surface of Fe_3O_4 microspheres by a one-step modification. Following that, a core–shell precipitation polymerization was carried out to create a molecularly imprinted shell on the Fe_3O_4@KH-570 microspheres by using MAA and *N*,*N*′-methylenebisacrylamide (MBA) as functional monomer and cross-linker, respectively. The structure and component of SIMs were well investigated by X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), thermogravimetric analysis (TGA), vibrating sample magnetometer (VSM) and Fourier transform infrared spectroscopy (FTIR). Most importantly, the adsorption isotherms, adsorption kinetics, effects of pH, specific selectivity and regeneration binding capacities of SIMs were also investigated in detail.