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Protein imprinted polymer using acryloyl-β-cyclodextrin and acrylamide as monomers

Wei Zhang [a], Lei Qin [a], Run-Run Chen [a], Xi-Wen He [a], Wen-You Li [a,*], Yu-Kui Zhang [a,b]

[a] Department of Chemistry, Nankai University, 94 Weijin Road, Tianjin 300071, China
[b] National Chromatographic Research and Analysis Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116011, China

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ABSTRACT

A novel protein imprinted polymer was prepared using acryloyl-β-cyclodextrin (β-CD) and acrylamide as monomers on the surface of silica gel. The bovine hemoglobin was used as template and β-CD was allowed to self-assemble with the template protein through hydrogen bonding and hydrophobic interaction. Polymerization was carried out in the presence of acrylamide as an assistant monomer, which resulted in a novel protein imprinted polymer. After removing the template, imprinted cavities with the shape and spatial distribution of functional groups were formed. Bovine serum albumin (BSA) cytochrome *c* (Cyt) and lysozyme (Lyz) were employed as non-template proteins to test the imprinting effect and the specific binding of bovine hemoglobin to the polymer. The results of the adsorption experiments indicated that such protein imprinted polymer, which was synthesized with β-CD and acrylamide as monomers, could selectively recognize the template protein.

1. Introduction

Molecularly imprinted polymer (MIP) was highly cross-linked polymer prepared by copolymerizing functional monomers and crosslinker in the presence of template molecule. After the template molecule was removed from the polymer, the complementary binding sites with specific recognition ability were created. The remarkable advantages of MIP compared to biosystem like antibodies are their reusability and low cost. To date, MIP has been widely used in the fields of chromatographic separation [1,2], as antibody mimetics and artificial receptor [3,4], and catalysis [5].

The majority of the MIP has been prepared using small molecules as template, however, imprinting against proteins was still a challenge [6–13], which is primarily due to the complexity of the protein structure and the variety of their sequence. Hjerten's group prepared polyacrylamide MIP with protein as template and the results of the experiment showed that the MIP could selectively recognize template protein [14,15]. Shiomi et al. [16] prepared protein imprinting polymer using covalently immobilizing template protein on the surface of silica. The results suggested that the imprinted cavities were successfully created on the silica. Highly selective recognition of protein could be achieved through a three-dimensional distribution of functional groups [17–19]. β-CD is an interesting molecule because it forms inclusion compounds with hydrophobic guest through hydrophobic interaction and hydrogen bonding [20]. Hjerten's group studied different interactions between β-CD and the proteins and the investigation showed that charged β-CD could affect the (capillary) electrophoretic separation of peptides and proteins [21]. The β-CD as monomer was successfully employed to small molecules [22–24]. Recently, Liu and coauthors [25] using β-CD as functional monomer successfully prepared bilirubin imprinted polymer and the MIP possessed high affinity and selectivity. Komiyama and coauthors reported that two kinds of modified β-CD monomers were applied to the imprinting toward amino acid derivatives and oligopeptides, and the MIP could selectively recognize the template molecules from mixture [26,27]. In our previous work [28], a novel molecularly imprinted polymer selective for tryptophan was fabricated with bonded β-CD and acrylamide (AA), and the results indicated that MIP using bonded β-CD and AA has higher imprinting effect to template molecule.

Synthetic protein imprinted polymer using acryloyl-β-CD and AA as monomers was prepared in the present work. Introduction of a large number of weak complementary interactions facilitated the protein recognition [24,29]. So the acryloyl-β-CD and AA cooperated together were considered for the improvement of the protein imprinted polymer performance. As shown in Fig. 1, the large number of possible bonds can be created between the protein and the functional monomers, and the spatial arrangement of the complementary functional entities of the network, together with the shape image correspond to the imprinted molecules.

* Corresponding author. Tel.: +86 22 2349 4962; fax: +86 22 2350 2458.
E-mail address: wyli@nankai.edu.cn (W.-Y. Li).

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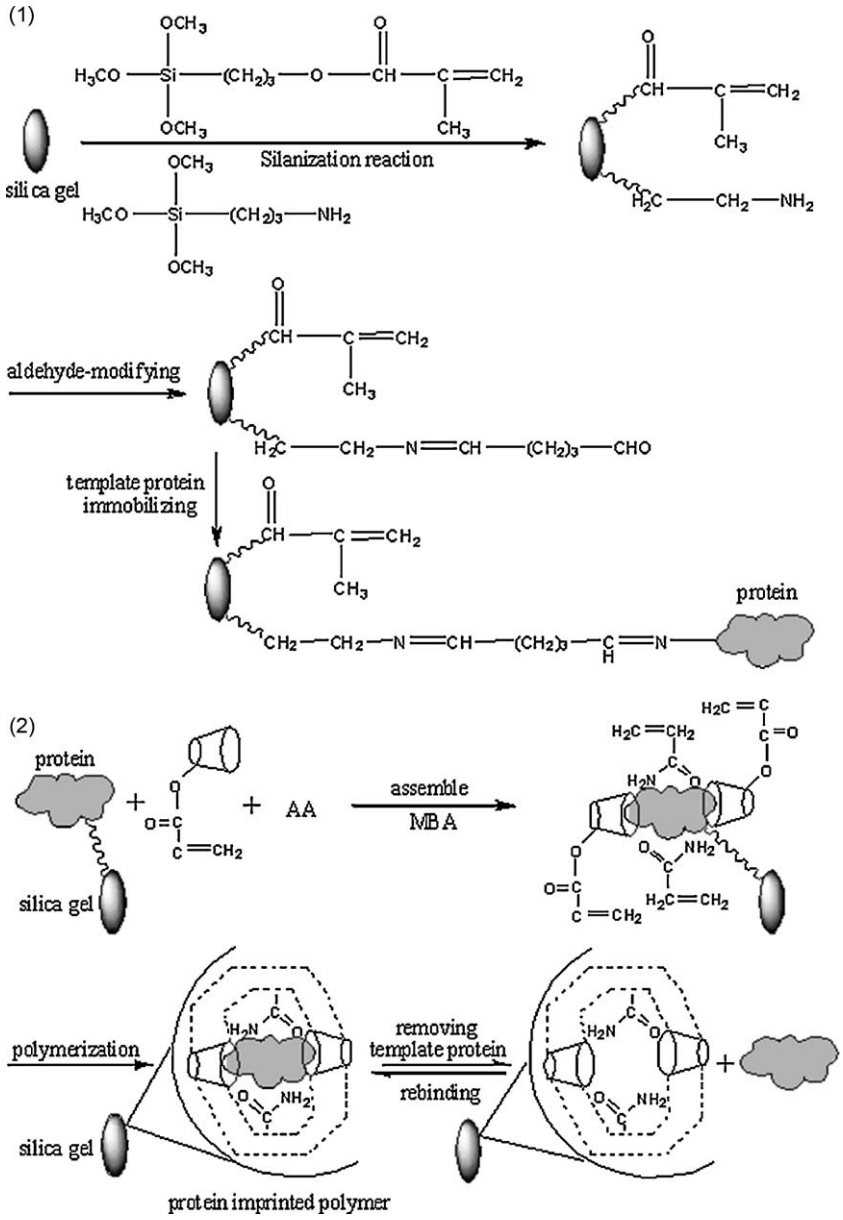

Fig. 1. The schematic representation for synthesis of the protein imprinted polymer.

Surface imprinting based on oriented immobilization of the template on silica gel, which facilitates the mass transfer of template protein, was employed in the present work. The bovine hemoglobin (BHb) was used as the template protein and was covalently immobilized on the surface of silica gel, and the MIP was formed on the surface of silica gel. And after the template protein was removed, complementary binding sites were thus created on the surface of silica gel. A series of adsorption studies were conducted and the results demonstrated that the MIP was capable of selective recognition of the template protein.

2. Materials and methods

2.1. Apparatus

UV-2450 UV–vis spectrophotometer was from Shimadzu (Kyoto, Japan). The surface morphology of the particles was studied using a Quanta 200 scanning electron microscope (FEI, Eindhoven, The Netherlands). Vario EL elemental analyzer (Elementar, Hanau, Germany) was employed to investigate the surface elemental composition of the particles. Fourier transform infrared (FT-IR) spectra (4000–400 cm^{-1}) in KBr were recorded on the AVATAR 360 FT-IR spectrophotometer (Nicolet, Waltham, MA, USA).

2.2. Materials and reagents

Silica gel of ultra pure (40–60 μm, 15 nm, Acros Organics, Geel, Belgium) was activated with acid before being silanized. Bovine serum albumin (BSA, molecular weight (MW) 67 kDa, isoelectric point (p*I*) 4.9), bovine hemoglobin (BHb, MW 66 kDa, p*I* 6.7), cytochrome *c* (Cyt, MW 12.4 kDa, p*I* 10.2) and lysozyme (Lyz, MW 14.4 kDa, p*I* 11) used in this study were purchased from LanJi of Shanghai Science and Technology Development Company (Shanghai, China). 3-Methylacryloxypropyl trimethoxysilane (WD-70) and 3-aminopropyl trimethoxysilane (WD-56) were purchased from Chemical Factory of Wuhan University (Wuhan, China). β-CD was from Institute of Tianjin JingKe Fine Chemicals (Tianjin, China). AA and N,N′-methylenebisacrylamide (MBA) were purchased from Chemistry Reagent Factory of Chinese QianJin (Tianjin, China).