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The effectively specific recognition of bovine serum albumin imprinted silica nanoparticles by utilizing a macromolecularly functional monomer to stabilize and imprint template

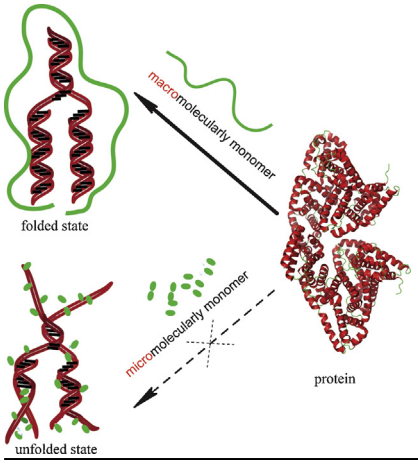
Liwei Qian *, Xiaoling Hu, Ping Guan, Dan Wang, Ji Li, Chunbao Du, Renyuan Song, Chaoli Wang, Wenqi Song

School of Natural and Applied Science, Northwestern Polytechnical University, The Key Laboratory of Space Applied Physics and Chemistry, Xi'an 710072, China

HIGHLIGHTS

- Bovine serum albumin was stabilized and imprinted by using a macromolecular chain.
- The sensitive monomer was used to prepare macromolecular chain.
- Surface imprinting of BSA by macromolecular chains on silica nanoparticles was prepared.
- The imprinted silica nanoparticles exhibited excellent selectivity and recognition ability.

GRAPHICAL ABSTRACT



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ABSTRACT

Structural stability of the template is one of the most important considerations during the preparation of protein imprinting technology. To address this limitation, we propose a novel and versatile strategy of utilizing macromolecularly functional monomers to imprint biomacromolecules. Results from circular dichroism and synchronous fluorescence experiments reflect the macromolecularly functional monomers tendency to interact with the protein surface instead of permeating it and destroying the hydrogen bonds that maintain the protein's structural stability, therefore stabilizing the template protein structure during the preparation of imprinted polymers. The imprinted polymers composed of macromolecularly functional monomers or their equivalent micromolecularly functional monomers over silica nanoparticles were characterized and carried out in batch rebinding test and competitive adsorption experiments. In batch rebinding test, the imprinted particles prepared with macromolecularly functional monomers exhibited an imprinting factor of 5.8 compared to those prepared by micromolecularly functional monomers with the imprinting factor of 3.4. The selective and competitive adsorption experiments also demonstrated the imprinted particles made by macromolecularly functional monomers possessed much better selectivity and specific recognition ability for template

* Corresponding author. Tel.: +86 13669272714.
E-mail address: qianliwei@mail.nwpu.edu.cn (L. Qian).

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protein. Therefore, using macromolecularly functional monomers to imprint may overcome the mutability of biomacromolecule typically observed during the preparation of imprinted polymers, and thus promote the further development of imprinting technology.

1. Introduction

Molecular imprinting is a promising technique for artificial synthesis of affinity recognition sites in polymeric matrices. Synthesis involves the co-polymerization of functional monomers and cross-linkers in the presence of a template molecule, which is subsequently removed to form the resulting recognition site imprinted polymers. Thus, the obtained molecularly imprinted polymers (MIPs) are complementary in shape, size and functionality with respect to the template. Compared with natural recognition materials like natural antibodies or ligand–receptor, MIPs offer advantages such as good mechanical and chemical stability, highly specific recognition and relative ease of mass preparation. They have therefore been applied in a wide variety of fields, including applications in biosensors, bioseparation, medical diagnostics and drug delivery [1–4]. To date, the molecular imprinting technology against small molecules has been well established. However, the imprinting of biomacromolecules, like protein, enzymes, DNA, viruses and even cells poses many challenges [5,6]. The major problem lies in the complex and flexible structures of these biomacromolecules, which can be easily denatured and unfolded during the MIPs preparation process [7]. Although some smart methods like micro-contact imprinting [8,9], were employed to avoid this problem by making the template an insusceptible protein crystal in the preparation of MIPs, lots of protein imprinted polymers in current researches nevertheless lack selectivity and specific recognition ability when compared to small molecule MIPs. Furthermore, numerous studies have reported the adverse impact of commonly used monomers, like acrylamide, methacrylic acid and acrylic acid, on the structural stability of proteins [10,11]. Therefore, how best to maintain protein structure during the preparation of MIPs, is a methodological problem that continues to be explored [12–14].

We speculate that the destructive effect of common monomers, here we call micromolecularly functional monomers (MIM), on the structural stability of protein, might be due to the nature of their small size, more easily allowing them to permeate template biomolecules and destroy the hydrogen bonds that maintain their native conformation. Therefore, based on this speculation, we propose a versatile and novel strategy to stabilize template proteins during the MIPs preparation by using inflexible macromolecularly functional monomers (MFM) to imprint biomacromolecules.

On the other hand, another difficulty regarding the use of macromolecules for imprinting applications lies in restricted mass transfer across the cross-linked polymer matrix due to their large molecular sizes, which restricts the ease of template removal as well as rebinding. To solve this limitation, several strategies have been developed including the epitope approach [12,13], surface imprinting technology [14–21] and adopting sensitive material in the preparation of MIPs [22–24].

In this work, we present a novel and versatile method to imprint and stabilize the template protein by using a MFM. The sensitive monomer, *N*-isopropylacrylamide (NIPAm), utilized to fabricate the MFM provides temperature-sensitivity in order to facilitate protein removal and rebinding. The resultant MFM and its equivalent MIM were compared in protein structural stability experiments via circular dichroism (CD) and synchronous fluorescence spectroscopy. In order to investigate the influence that the structural stability of the template protein has on selectivity of MIPs, non-imprinted polymers (NIPs) and MIPs were prepared by using MFM or its equivalent MIM on the surface of silica nanoparticles. The resultant imprinted nanoparticles were evaluated by investigating adsorption dynamics, adsorption isotherm and competitive adsorption experiments.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS), γ-methacryloxypropyltrimethoxysilane (MPS), 1-(chloromethyl)-4-ethenyl-benzene (CMS) and 1-vinylimidazole (VIM) were obtained from Sigma (St. Louis, MO, USA). *N*-Isopropylacrylamide (NIPAm) was supplied by Acros Organics (Morris Plains, NJ, USA). Ammonium persulfate (APS) and *N*,*N*,*N*,*N*-tetramethylenebis(acrylamide) (TEMED) were provided by Sigma–Aldrich (Tokyo, Japan). Bovine calf serum (BCS), bovine serum albumin (BSA), bovine hemoglobin (Hb), ovalbumin (OVA) and lysozyme (Lyz) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and used as received.

2.2. Characterization

Fourier transforms infrared (FTIR) spectra were acquired on a TENSOR27 FTIR spectrometer (Bruker). The samples were prepared by mixing the products with KBr and pressing into a compact pellet. Morphology and structure of the nanoparticles were observed using transmission electron microscopy (TEM, JEOL JEM-3010). The polymer content of these nanoparticles was determined by thermogravimetric analysis (TGA, Q50, TA instruments) in the temperature range of 100–700 °C, with a heating rate of 10 °C min^{-1} under nitrogen atmosphere. Dynamic laser light scattering (DLS) was performed on a modified commercial laser light scattering spectrometer (ALV SP125) equipped with an ALV-5000 multi digital time correlator and a He–Ne laser (output power = 10 mW, λ = 632 nm). The absorption spectra were measured using a UV spectrophotometer (Varian, Cary-1E). The CD experiments were carried out using an applied photophysics Chirascan™ instrument at 30 °C with a 1 cm quartz cuvette. Fluorescence measurements were performed using a Hitachi F-4500 spectrofluorometer at 30 °C with a 1 cm quartz cuvette. The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded by using an Avance 300 MHz spectrometer. X-ray photoelectron spectroscopy (XPS) was carried out using an Axis Ultra DLD spectrometer (Kratos) to determine the chemical composition. The relative molecular weight of MFM and its distribution was determined using Waters 150C gel permeation chromatography (GPC) equipped with Waters 1515 HPLC pump, Waters 2414 differentia refractive index detector and Ultrastyragel columns at 30 °C. Element analysis was carried out using the Vario EL cube elemental analyzer (Elementar, Germany). Protein identification was performed using a Shimadzu LC-2010A series HPLC (Japan) with an Xtimate SEC (250 mm × 7.8 mm, 5 μm, 300 Å) column. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a DYY-6C electrophoresis system (Beijing Liuyi instrument plant).