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Molecularly imprinted polymers based electrochemical sensor for bovine hemoglobin recognition

Xianwen Kan*, Zonglan Xing, Anhong Zhu, Zhe Zhao, Guilin Xu, Chen Li, Hong Zhou

College of Chemistry and Materials Science, Anhui Key Laboratory of Chemo-Biosensing, Anhui Normal University, Wuhu 241000, PR China

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ABSTRACT

A simple and efficient molecularly imprinted polymers (MIPs) based electrochemical sensor were proposed and prepared by electropolymerization of pyrrole in the presence of bovine hemoglobin (BHb) in an aqueous solution. The fabrication process of the sensor was characterized by differential pulse voltammetry and electrochemical impedance spectroscopy, in which $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ was used as an electrochemical active probe. The influence factors including electropolymerization cycles, scan rate, the concentration of pyrrole, and the extraction conditions were investigated in detailed. Under the optimized conditions, the experimental results showed that the MIPs based sensor possessed a fast rebinding dynamics and an excellent recognition capacity to BHb, compared to the other non-template proteins. Moreover, the prepared sensor also exhibited a dependent relationship between the concentration of template protein and peak current of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$.

1. Introduction

Affinity matrixes for the separation of proteins are highly desirable in biotechnology for separation and purification, in the field of diagnostics, and in biosensor technology [1,2]. However, as an important property for a biomimetic receptor system, the selectivity of these matrixes is not as high as antibody-based system. Thus, the design and construction of the biomimetic receptor system with high selectivity has attracted much more attention. Molecularly imprinting, as one of the most promising techniques for the formation of affinity recognition sites, has been proposed and developed very fast in recent years [3–5]. The affinity matrix is prepared by the polymerization of functional monomers and cross linkers in the presence of template molecules. Subsequent removal of the template molecule from the polymer, molecularly imprinted polymers (MIPs) is obtained with the complementarity in shape, size and functional groups to the template molecule, which could selectively recognize template molecule. Advantages including chemical and thermal stability, reusability, cost-effective fabrication, and high recognition capacity to the template molecule enable MIPs to be applied in a variety of areas, such as chiral separation, solid phase extraction, biosensors, and drug controlled release [6–10]. So far, MIPs have been successfully developed against a wide range of small molecules recognition and detection [11–13]. Meanwhile, biomacromolecules (including protein, virus, and cell) imprinting is also arousing researchers more and more attentions and interests to meet the requirement in the field of macromolecules separation and detection [14,15].

However, protein imprinting is still a challenge currently because of the following limitations. First, it is difficult for template protein to be extracted from a bulk polymers matrix completely after the polymerization as well as to be rebound into the imprinted sites. Second, organic solvents, generally for the preparation of MIPs, are not suitable for the protein MIPs synthesis due to the unstable three-dimensional conformations, possible rearrangement processes and poor solubility of the protein molecule in organic solvents. Third, the large size and structural complexity of the protein leads to more non-specific rebinding and poor recognition behavior by the MIPs [16]. In spite of these difficulties, there is still a strong attempt to prepare protein imprinted polymers for the use in bioenrichment, bioseparation, biosensors, and so on [17–20]. And more and more new strategies and materials were proposed to prepare MIPs for protein recognition. A thermosensitive macroporous hydrogel showing selectivity for lysozyme was developed based on metal coordination interaction by Zhang et al. [21] The interaction of the imprinted hydrogel to the template protein could be switched between the coordinate effect and the electrostatic effect by adding or not adding Cu ions. Zhao and co-workers reported a novel stimuli-responsive protein imprinted polymer for selective recognition of bovine serum albumin by using N-isopropylacrylamide and N-[3-(dimethylamino)propyl]-methacrylamide as functional monomers [22]. Among the numerous MIPs, the most effective way of these MIPs should be produced by surface imprinting technique. This

* Corresponding author. Tel.: +86 553 3937135; fax: +86 553 3869303.
E-mail address: kanxw@mail.ahnu.edu.cn (X. Kan).

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approach prepares the polymers with imprinted sites located at or close to the surface of the MIPs, enabling easy extraction and rebinding of the template protein.

The polymerization of the 2-dimensional MIPs thin film on electrode surface to construct a MIPs based electrochemical sensor has been widely performed for the recognition and detection of the template molecules [23–25]. In electrochemical sensors, MIPs can either accumulate template molecules on the electrode surface to enhance the sensitivity of the sensor or separate template molecules from the other analytes to improve the selectivity of the sensor. The literatures on MIPs for protein recognition and detection were compounded by the numerous methods proposed for the transduction of the binding event to a useful signal, such as the change of frequency, potential, and current [26,14,27]. Dickert et al. [28] developed another method for viruses and proteins recognition by self-assembly of template molecules on a stamp to generate patterns on a polymer. They demonstrated that the geometrical fit and noncovalent interactions were the main reasons for the function of recognition. Viswanathan et al. [29] prepared protein imprinted polymer on three-dimensional gold nanoelectrode to detect epithelial ovarian cancer antigen-125 (CA 125). Electrochemical experimental results revealed that the sensor had good selectivity and sensitivity for template molecule. And the sensor showed good increments at the concentration range of 0.5–400 U/mL for CA 125. A potentiometric protein sensor was also reported on the surface molecular imprinting technique by Rafailovich et al. [30]. The imprinted sensing layer was fabricated by self-assembling of alkanethiol monolayers in the presence of myoglobin or hemoglobin, which could recognize the template protein from other protein molecules. Among these MIPs based electrochemical sensors, amperometric sensors were reported to successfully recognize and detect protein [27,31,32]. Cai et al. [31] developed molecularly imprinted sensors specific for human ferritin and human papillomavirus derived E7 protein. The synthesized imprinted non-conducting polymer on carbon nanotube tips showed subpicogram per liter sensitivity by electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). Wu et al. [32] fabricated a hemoglobin imprinted polymers based electrochemical sensor by cyclic voltammetry method. The prepared sensor exhibited a good recognition capacity and a good response in the range of low concentration from 0.00 to 1.00 mg/mL for hemoglobin.

Polypyrrole, as one of the best candidates for the electrochemical sensor preparation, as well as the good biocompatibility and the easy ways for the immobilization of biomolecules, has been employed to fabricate MIP-based sensor for a variety of molecules recognition and detection [33–36]. In this study, a simple and efficient MIPs based electrochemical sensor were proposed and prepared by electropolymerization of pyrrole in the presence of bovine hemoglobin (BHb) in the aqueous solution. The fabrication process of the sensors was characterized by differential pulse voltammetry and electrochemical impedance spectroscopy. Under the optimized conditions, the experimental results showed that the MIPs based sensor possessed a fast rebinding dynamics and an excellent recognition capacity to BHb.

2. Experimental

2.1. Chemicals

Bovine hemoglobin (BHb), bovine serum albumin (BSA), lysozyme (Lyz), and albumin egg (EA) were purchased from Sigma. Pyrrole was obtained from Fluka (Fluka Chemie AG, Switzerland). All other reagents used were of AR grade and used as received without further purification. All solutions were prepared with double-distilled deionized water.

2.2. Apparatus

Electrochemical experiments, such as cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) were performed on a CHI 660C workstation (Chenhua Instruments Co., Shanghai, China) with a conventional three-electrode system. A bare or modified Au electrode (AuE) served as the working electrode, and a saturated calomel electrode and a platinum wire electrode were used as the reference and counter electrodes, respectively. The actual pH values were determined with a pH/Ion Analyser model pHS-3CT (Da Pu Instrument Co., Ltd., Shanghai, China). The extraction of template protein was carried out in a thermostat steam bath vibrator (Jite Instrument Co., Ltd., Jiangsu, China). Field emission scanning electron microscope (FE-SEM) images were obtained on an S-4800 field emission scanning electron microanalyser (Hitachi, Japan).

2.3. Fabrication of the MIPs modified Au electrode (MIPs/AuE)

An Au electrode was polished with alumina powder (1.0 and 0.05 μm) and sonicated in ethanol and water for 3 min. Then, the electrode was electrochemically cleaned by consecutive cycling in 0.5 mol/L H_2SO_4 between −0.20 and 1.60 V at 100 mV/s until a characteristic cyclic voltammogram of a clean Au electrode was obtained. The obtained electrode was dried under pure nitrogen flow for the further use.

The clean electrode was immersed into a deoxygenated aqueous solution containing 5 mmol/L pyrrole, 0.1 mol/L KCl, and 1 mg/mL BHb and the electropolymerization was carried out using CV method at a rate of 100 mV/s between −0.2 and +1.2 V for 6 cycles to give a polymer modified AuE. After the electropolymerization, the polymers modified electrode was incubated into a solution of 1 mol/L H_2SO_4 for 2 h to extract embedded template protein. Then the modified electrode was immersed into PBS (0.1 mol/L, pH 7.0) for scanning between −0.6 and +1.00 V for several cycles to overoxide the polypyrrole matrix, obtaining MIPs/AuE.

As a control, a non-molecularly imprinted polymers (NIPs) modified Au electrode (NIPs/AuE) was also prepared and treated in exactly the same manner, except for the omission of BHb in the electropolymerization process.

The procedure for the preparation of the MIPs/AuE is depicted in Scheme 1.

2.4. Electrochemical property measurements

$[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ was chosen as an electrochemical active probe to study the performances of the prepared sensor due to the poor electroactivity of BHb. Imprinted cavities formed in the MIPs could provide pathways for the diffusion of probe into and out of the MIPs matrix, which then is oxidized or reduced at the electrode and produce an electrochemical signal. Therefore, all the electrochemical experiments were carried out in PBS containing 10 mmol/L $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ by using different electrochemical techniques, including CV, DPV, and EIS to investigate the changes of electrochemical signals of the probe. BSA, Lyz, and EA were selected as the comparative proteins to evaluate the recognition capacity of the prepared sensor.

3. Results and discussion

3.1. Characterization of the MIPs/AuE

The morphologies of MIPs/AuE and NIPs/AuE were detected by SEM, as shown in Fig. 1. It could be seen from the SEM images that there was great difference in the morphologies between MIPs/AuE and NIPs/AuE. MIPs film formed on AuE surface was remarkably