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Highly sensitive protein molecularly imprinted electro-chemical sensor based on gold microdendrites electrode and prussian blue mediated amplification

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ABSTRACT

In this paper, a novel strategy of protein molecularly-imprinted electrochemical sensor was proposed to determine Bovine Hemoglobin selectively. Gold microdendrites and prussian blue particles were electrodeposited on the surface of glassy carbon electrode respectively to improve the sensitivity of MIECS. The molecularly imprinted polymers film is synthesized by electrochemically induced redox polymerization of acrylamide in the presence of template protein. The surface feature of the modified electrode was characterized by cyclic voltammetry. The reduced electrochemical intensity which was produced by prussian blue is related to the concentration of Bovine Hemoglobin. The times of washing and incubation of modified electrode were investigated and optimized. The MIECS with improved conductivity and electrochemical performances showed good response in the range of low concentrations from 0.1 to 1.0×10^3 μg/mL and the detection limit (LOD, 3σ, RSD $\leq$ 4.31%) is 0.05 μg/mL. The result demonstrates significant specific recognition toward the template protein via selective test and a reasonable regeneration in five recyclings. The proposed sensor exhibited high sensitivity and selectivity, acceptable reproducibility, and could be extended to recognize other protein.

1. Introduction

Molecularly imprinted electrochemical sensor (MIECS) combined the strong points of both molecularly imprinted polymers (MIPs) and electrochemical sensors (Haupt and Mosbach, 2000), demonstrates high selectivity, chemical and mechanical stability, durability, reusability, ease of preparation, low cost, and so on. Due to these special advantages MIECS have obtained wide applications in medical, biological and environmental analysis (Suryanarayanan et al., 2010).

Although research in MIECS has been successfully used to recognize various molecules(Xing et al., 2012; Pan et al., 2011; Liang et al., 2010), imprinting of large structures, such as biomacromolecules, in particular proteins, is still a challenge (Whitcombe et al., 2011; Janiak and Kofinas, 2007). Due to the properties of proteins, such as the large molecular size, flexible conformation, and large number of functional groups available for recognition (Levi and Srebnik, 2010; Bossi et al., 2007) new strategies have been proposed to address the intricacy of protein MIECS. The main methods include the use of polymeric materials (Chen et al., 2012a, 2012b; Balogh et al., 2011; Reddy et al., 2011) and modification of electrode (Zhu et al., 2011; Díaz-Díaz et al., 2012) to enhance the sensitivity and selectivity of the electrochemical sensor. The choice of polymeric materials is the most important aspect in imprinting the template molecule. The biocompatible acrylamide system has been widely investigated and successfully applied in protein imprinting (Li et al., 2006; Wu et al., 2010, 2012) because it can be polymerized in aqueous environments.

Extensive investigations in MIECS have been reported for the determination of protein. A multiwalled carbon nanotubes-ceramic electrode modified with a new substrate-selective imprinted polymer was developed for ultra-trace detection of bovine serum albumin as low as 0.42 ng/mL by Prasad et al. (2013). Viswanathan et al. (2012) developed protein imprinted polymer on three-dimensional gold nanoelectrode ensemble to detect epithelial ovarian cancer antigen-125 (CA 125) with the lowest detection limit as 0.5 U/mL, but the assembly of electrode was relatively difficultly to operate. Bovine Hemoglobin (BHb) is well known for its function in the vascular system of animals, being a carrier of oxygen. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood (Scheller et al., 2005).

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Some researches were proposed to prepare MIPs for BHb recognition (Kan et al., 2012, Wu et al., 2010). Among the numerous MIECS, the most effective way should be produced by surface modified technique which can improve the conductivity and increase the surface area.

In recent years, gold nanostructures have stimulated great interest due to their high conductivity, chemical and thermal stability, and promising applications in nanoelectronics, biomedicine, sensing, and catalysis (Tikariha et al., 2012; Huang et al., 2010). The gold nanostructures-modified electrode can be easily constructed by some strategies including direct electrostatic assembly, covalent linking, polymer entrapment or co-mixing, sol–gel, and electrodeposition methods(Huang et al., 2010; Guo et al., 2011; Tang et al., 2012; Li et al., 2011; Lin et al., 2011; Zhang et al., 2004). Due to their unique physical and chemical features of three-dimensional nanostructures, gold dendrites have attracted a great deal of attention with a wide application in electrochemical sensors. They can provide large active surface, which is promising to increase functional density and facilitate electron-transfer, resulting in several orders of magnitude in electrochemical signals. The development of hierarchical gold nanostructures by simple electrodeposition method is very attractive for the applications in biosensor, electrocatalysis, etc.

Prussian blue (PB) $[Fe_4[Fe(CN)_6]_3]$ has been considered an "artificial enzyme peroxidase" because it shows high selectivity and catalytic activity for the reduction of H_2O_2 (Chen et al., 2012a, 2012b). More importantly, as an inorganic conductive film, a PB film can replace electronic media to directly produce electrochemical signals to improve sensitivity. PB has been widely used as a mediator in electrochemical biosensors (Bustos and Godínez, 2011), but PB-based molecular imprinting technique is rarely reported.

Herein, we present a technique for the preparation of MIECS based on gold dendrites and PB film modified electrode to enhance MIECS sensitivity by electrochemically-induced copolymerization of acrylamide and *N*,*N*′-methylene diacrylamide in the presence of BHb template molecules. The MIECS can selectively recognize the template molecule. The adsorbed BHb is detected by electrochemical signal reduction of PB due to electrochemical inert of protein impending electron transfer of PB at the electrode surface. The electrochemical signal intensity is related to the concentration of BHb. The result shows a wide detection range, excellent selectivity and reproducibility, satisfactory stability, low cost, and easy preparation of the MIECS.

2. Experimental

2.1. Materials

Phosphate buffered saline (PBS, 0.01 M, pH 7.4), Acrylamide (AAm) and *N*,*N*-methylene-bisacrylamide (MBA) of electrophoresis grade, Ovalbumin(OVA), Bovine hemoglobin (BHb), Cytochrome c (Cyt c), and bovine serum albumin (BSA) were purchased from Sigma. Gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), ammonium persulfate (APS), Hydrochloric acid (HCl), ferric chloride ($FeCl_3$), KCl, potassium ferricyanide $[K_3Fe(CN)_6]$ of analytical grade were received from Xinyuhua (Fuzhou, China). Milli-Q purified water was used for all experiments described here.

2.2. Apparatus

The electrochemical measurements were performed with a CHI 660D electrochemical workstation (Shanghai, China). The classical three-electrode system consisted of an MIP-modified glass carbon electrode (GCE) (3 mm diameter) as the working electrode, a potassium chloride (KCl)-saturated calomel electrode (SCE) as the reference electrode, and a Pt wire electrode as the auxiliary electrode. All electrochemical experiments were performed under a dry nitrogen atmosphere at room temperature.

2.3. Preparation of PB/DenAu(dendritic Au) modified GCE

Before modification, The GCE was polished with 0.3 and 0.05 μm alumina slurry, and rinsed thoroughly with doubly distilled water between each polishing step. Then it was sonicated in distilled water and ethanol for 5 min, and dried in air. A layer of hierarchical dendritic gold microstructures (HDGMs) was electrochemically deposited onto the cleaned GCE under constant potential electrolysis of −0.6 V for 600 s in the solution containing 0.1 M Na_2SO_4 and 30 mM $HAuCl_4$ according to the literature(Tang et al., 2012; Ye et al., 2010). The HDGMs electrode was inserted into an aqueous mixture consisting of 3.0 mM $K_3Fe(CN)_6$, 3.0 mM $FeCl_3$, 0.1 M KCl and 0.1 M HCl, then a constant potential of −0.40 V was applied for 600 s (Chen et al., 2012a, 2012b). Subsequently, the PB/DenAu GCE was rinsed with distilled water and immersed into a solution containing 0.1 M KCl and 0.1 M HCl for cyclic voltammetric sweep in the potential region of 0 and 0.35 V at a scan rate of 50 mV s^{-1}, until a stable response was obtained.

2.4. Preparation of MIP and non-MIP (NIP) modified Sensors

The BHb MIP membrane is fabricated through the electrochemical copolymerization. The electropolymerization is performed by CV (five cycles) (Wu et al., 2010) in the potential range from −1.2 to −0.4 V with a scan rate of 20 mV s^{-1}. The polymerization solutions contain 22.5 mg AAm (functional monomer), 3 mg MBA (crosslinking agent), 2 mg APS (catalyst), 5 mg BHb (template) in 1 mL PBS (0.01 M, pH 7.4). High-purity nitrogen was bubbled at least 10 min to remove dissolved oxygen before electropolymerization. Similarly, the non-MIP is also fabricated in the absence of BHb. After that, the electrode was extracted with 10% (v/v) acetic acid containing 10% (w/v) SDS solution for 60 min to remove the template protein, then washed with doubly distilled water, and saved in PBS. The schematic representation is illustrated in Scheme S1 (see Supporting Information). The electrodeposited PB served as an electrochemically 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 PB electron transfer in the surface of modified electrode which then produced a change of electrochemical signal. Therefore, the proposed MIECS were carried out in 0.1 M KCl which acted as supporting electrolyte.

3. Results and discussion

3.1. Morphological characterization of the MIP PB/DenAu/GCE

The surface morphologies of the prepared different modified films were studied by means of SEM. Fig. 1A and D displays the SEM image of HDGMs modified interface. A layer of three-dimensional HDGMs could be observed, which was in accordance with the reported result (Tang et al., 2012). The hyperbranched structures largely increased the surface coverage of the electrode, and hence the amount of effective binding sites. The PB deposited on the HDGMs (Fig. 1B and E) is well distributed with a rough surface. PB particles covered up most of the HDGMs and blurred the dendritic structure. The rough surface provided a large surface area for template molecular imprinting. The morphologies of MIP sensor were shown in Fig. 1C and F. A sol–gel layer was clearly visible on the rough surface. The experimental results indicated