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Sensors and Bioselective Reagents

Label-free amperometric immunosensor based on prussian blue as artificial peroxidase for the detection of methamphetamine

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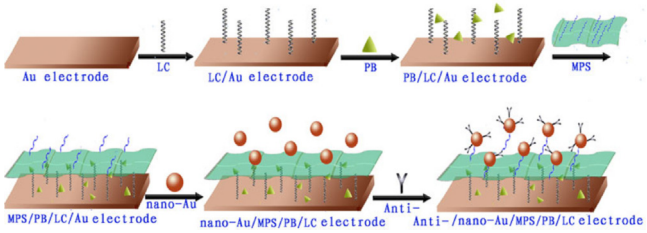
HIGHLIGHTS

- An amperometric immunosensor was developed for the detection of MA.
- PB could catalyze the reduction of H_2O_2 to amplify the amperometric signal.
- The immunosensor exhibited high selection, stabilization and sensitivity.

GRAPHICAL ABSTRACT

A LC film was assembled on the Au electrode by immersing the bare gold electrode in LC. The PB film was electrodeposited on the LC-modified electrode. Subsequently, MPS hydrolyzed and condensed was dropped onto the PB/LC electrode to form MPS/PB/LC-modified electrode. After that, the electrode was immersed in a solution of nano-Au colloid, anti-MA and BSA in turn.



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ABSTRACT

A label-free amperometric immunosensor for the detection of methamphetamine was developed. The prussian blue deposited/L-cystine-modified electrode was covered with nano-Au/(3-mercaptorpropyl) trime-thoxysilane film. Then, the nano-Au was used for the immunosensor platform to capture a large amount of anti-methamphetamine. PB exhibited excellent electrocatalytical properties toward the reduction of H_2O_2 at low overpotentia to amplify the amperometric signal, which enhanced the sensitivity of the immunosensor. The active sites of PB could be shielded and the access of H_2O_2 from solution to the electrode might be partially blocked after the completion of immunoassay, led to a linear decrease in the response current of the electrode over the range from 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} of MA. The obtained immunosensor displayed excellent catalytic reduction toward H_2O_2 due to high activity and selectivity of PB. The influence of relevant experimental variables, including the construction of immunosensor platform, the amount of MPS and the time of immunoaction, was examined and optimized.

1. Introduction

The drug abuse and smuggling of methamphetamine (MA) have increased year by year in the world though it has been strictly controlled, which can bring significant impacts on human health and social security. Therefore, the detection of trace MA is given a pressing concern for combating criminals and maintaining social stability. The typical transduction techniques for the detection of MA and related compound involve thin-layer chromatography [1], high-performance liquid chromatographic, gas chromatographic [2], high-performance liquid chromatographic–mass spectrometric [3,4] and gas chromatographic–mass spectrometric [5]. Recently, the quantitative analysis of MA is carried out with fluorescence [6,7] and chemiluminescence, especially electrochemiluminescence [8,9], which have been reported because of

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their high sensitivity, fast response and low-cost. The low selectivity, however, is hardly avoidable since these methods often suffer from the interference of non-specific reaction from real sample.

Conventional immunochemical analyses, based on specific interaction between an antibody and a complementary antigen, have become a powerful analytical tool for biochemical analyses, clinical diagnoses and environmental test. One of the most frequently used methods is enzyme-linked immunosorbent assay (ELISA), where enzymes are used as labeling for signal amplification of interaction between antigen and antibody [10]. Amperometric immunosensors, combining ELISA with electrochemical transduction, have attracted growing attention in recent years due to its highly sensitivity and selectivity compared with conventional immunoassay techniques. Sandwich and competitive assay are two basic techniques used in the amperometric immunosensors. In these cases, after the specific binding of antigen and labeled antigen to antibody immobilized on electrode, the electrochemical signal is amplified by the labeled enzyme in the presence of the enzyme substrate and it will determine the analyte concentration [11–13]. However, a lot of reductants in biological liquids can produce high noise signal because they are able to be oxidized. To avoid high noise signal in the amperometric immunosensor system, mediators have been developed to shuttle electrons between the enzyme and the electrode. Thus, the labeling amperometric immunosensors exhibit some disadvantage factors such as the loss of mediators, time-consuming and more reaction steps.

We acknowledge that the amperometric immunosensor for the detection of MA has not been well studied. In this work, a label–free electrochemical immunoassay based on anti-MA/nano-Au/MPS/PB/LC-modified electrode was developed for the detection of MA. The immunosensor showed a variety of advantages: (1) PB deposited onto the LC-modified electrode played two roles. First, it was used as an electron transfer mediator to enhance the analytical performance of electrochemical sensors; second, as the "artificial peroxidase", it could catalyze the reduction of H_2O_2 to amplify the amperometric signal of the immunosensor. (2) The PB layer was embedded the LC film across the spacing of the alkyl chain of LC, which improved adherence of the PB layer to the electrode. Moreover, the MPS hydrolyzed and condensed was dropped on the surface of the PB/LC-modified electrode, forming a stable film that protected the PB film against leaking. Both of the LC and MPS film enhanced the stability of the immunosensor. (3) The abundant thiol groups of MPS served as binding sites for nano-Au immobilization. Then, the nano-Au particles were used for the immunosensor platform to capture a large amount of antibody that improved the sensitivity and selectivity of immunosensor. The obtained immunosensor displayed excellent catalytic reduction toward H_2O_2 due to high activity and selectivity of PB. The active sites of PB on the immunosensor could be shielded and the access of its substrate to the electrode might be either partially or completely blocked as the specific binding of anti-MA to MA, which led to a liner decrease in amperometric signal over the range from 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} of MA with a detection limit (S/N = 3) of 7.5×10^{-9} mol L^{-1}. The label-free amperometric immunosensor not only displayed high selectivity, stability and sensitivity, but also decreased the analytical time and reaction steps.

2. Materials and methods

2.1. Materials

Antibody-methamphetamine (Anti-MA, 2.01 mg mL^{-1}) was purchased from GenWay Biotech, Inc. Methamphetamine·HCl (MA) was obtained from National Drug Lab (China). $NaBH_4$, bovine serum albumin (BSA) and (3-mercaptorpropyl) trimethoxysilane (MPS, 95%) were purchased from Sigma. L-cysteine (LC) was obtained from Kangda Amino Acid Reagent Co, Shanghai, China. $HAuCl_4$, $K_4Fe(CN)_6$, $FeCl_3$, were obtained from Chemical Reagent Co, Sichuan, China; and all other chemicals were of reagent grade and used as received. Phosphate-buffered saline (PBS) was used for the preparation of buffers.

2.2. Apparatus

Electrochemistry was performed using a CHI 660A electrochemical workstation (Shanghai CH Instruments Co., China) in a three-electrode electrochemical cell containing a platinum wire auxiliary electrode, a modified Au electrode as working electrode and a saturated calomel electrode (SCE) as reference electrode against which all potentials were measured. The measurements were performed in 25 mmol L^{-1} PBS (pH = 6.5). The field emission electron microscopy (SEM) of the particles was obtained in a field emission scanning electron microscopy (FE SEM LEO 1530). Raman spectra were obtained using a Renishaw Raman system model 2000 spectro-meter.

2.3. Electrode modification

The nano-Au was prepared by a method described in the previously reported literature [14]. MPS was hydrolysed and condensed according to the method in the literature [15]: 0.06 mL of MPS was added into the solution containing 1.0 mL of ethanol and 0.26 mL of 25% HAC to hydrolyze and condense by sonicating in an ice bath for 30 min. Gold disk electrode (2 mm in diameter) was carefully polished with alumina slurry (0.3 μm and 0.05 μm) and then ultrasonically cleaned in distilled water and ethanol. The preparation of the working electrode is depicted in scheme Fig. 1. Briefly, LC film was assembled on the Au electrode by immersing the bare gold electrode in 25 mmol L^{-1} PBS pH 6.5 containing 20 mmol L^{-1} LC [16,17], and then electrodeposition of the prussian blue (PB) film on the LC-modified electrode was accomplished in the solution of 2.5 mmol L^{-1} $K_4Fe(CN)_6$ + 2.5 mmol L^{-1} $FeCl_3$ + 0.1 mol L^{-1} KCl + 0.1 mol L^{-1} HCl by applying electrochemically cycled voltage between +350 and −50 mV. Subsequently, MPS hydrolyzed and condensed (1.5 μL) was dropped onto the PB/LC electrode and dried in air. Then the MPS/PB/LC electrode was immersed in nano-Au for 4 h. After that, anti-MA/nano-Au/MPS/PB/LC electrode was accomplished through immersing nano-Au/MPS/PB/LC-modified electrode in the anti-MA. Finally, the surface of antibody modified electrode was treated with BSA to block unspecific sites.

2.4. Amperometric measurement

Unless otherwise stated, the immunosensor was incubated in PBS (25 mmol L^{-1} pH 6.5) containing different concentrations of MA at 25 °C for 35 min, and then the immunosensor was transferred into 2 mL 25 mmol L^{-1} PBS (pH 6.5) containing H_2O_2 (25 mmol L^{-1}) to measure the amperometric response.

3. Results and discussion

3.1. Characterization of anti-MA-HR/nano-Au/MPS/PB/LC electrode

Cyclic voltammogram (CV) has been widely used to characterize electrochemical behavior of the preparation process of the working electrode because the electron transfer between the solution species and the electrode must occur by tunneling through the defects in the barrier. Fig. 2 shows the CVs of the different electrodes in PBS. Compared with that of LC electrode