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A label-free electrochemical immunosensor based on gold nanoparticles for direct detection of atrazine

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ABSTRACT

A simple, selective, highly sensitive, and label-free electrochemical immunosensor for atrazine detection was developed by immobilizing gold nanoparticles (GNPs) on the gold electrode surface. The modified gold electrode exhibited good electrochemical activity. The high sensitive detection was achieved by increased the surface area of work electrode resulting in capturing more anti-atrazine monoclonal antibodies. The whole immunosensor fabrication process was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) with the use of ferricyanide as an electrochemical redox indicator, respectively. The signal was triggered based on the interaction between anti-atrazine monoclonal antibody and atrazine via differential pulse voltammetry (DPV). Under the optimal conditions, a limit of detection for atrazine as low as 0.016 ng/mL was obtained in buffer, and the linear working range was between 0.05 ng/mL and 0.5 ng/mL. The recovery of 95.5–119.8% was obtained in maize. The proposed immunosensor has acceptable stability and reproducibility, thus could be applied to other pesticides analysis of crop samples in practice.

1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, ATZ) is one of the most broadly used pesticides in agriculture. Due to its persistence, high mobility and slow degradation rate, atrazine are commonly found in soil, surface water and agriculture food products. When human are exposed to atrazine, it will have an effect on human's reproductive system, endocrine system, central nervous system and the immune system [1–3]. Therefore, it becomes necessary to detect the atrazine in crop, drinking water and soil.

Currently, the determination methods for atrazine mainly rely on high performance liquid chromatography (HPLC), mass spectrometry (MS), LC–MS/MS, and gas chromatography–mass spectrometry (GC–MS) [4–11]. Although these techniques may provide efficient determination, they are quite laborious, time-consuming, and require large volume of sample as well as highly-trained personnel. Recently, the application of enzyme-linked immunoassays (ELISA) [12,13] and immunosensor have attracted considerable attention in detection atrazine owing to their intrinsic advantages, such as high sensitivity, good specificity, less dependence on sample pretreatment and high compatibility with progressive micromachining technologies. The immunosensor is different from immunoassay [14]. They unite the specificity of the antibody–antigen immunoreaction with fast, often direct, data acquisition possible with immunosensor processes. Several sensors for atrazine have been evaluated, such as electrochemical immunosensor [15–17], electrochemical sensor based on molecular imprinted polymers [18], surface plasmon resonance (SPR) immunosensor [19–21], fluorescent and chemiluminescence immunosensor [22].

The key issue with any immunosensor is reliable immobilization of a high density of antibodies with simple immobilization process, and moreover maintains bioactivity of the antibodies for immunoreaction. Over the years, gold nanoparticles (GNPs) have attracted much attention as a sensing platform in electrochemical immunosensor because of their distinctive advantages, such as easy preparation, high specific surface area, good biocompatibility and high electrical conductivity [23–25]. GNPs as a sensing platform not only can increase the amount of immobilized antibodies, but also can accelerate the electron transfer process for signal enhancement. Moreover, the electrocatalytic action of GNPs could facilitate low-potential amperometric measurement of the electrochemical immunosensor. There have been some reports on

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the application of GNPs as sensing platforms in electrochemical immunosensor development. Lu et al. [26] fabricated a human chorionic gonadotrophin (hCG) electrochemical immunosensor with layer-by-layer assembled films of GNPs, graphene nanosheets, and multiwalled carbon nanotube (MWCNTs). Jeong et al. and Liu et al. constructed GNPs electrode for rapid and sensitive detection of carcinoembryonic antigen and ochratoxin A, respectively [27,28].

In this work, we show a simple electrochemical immunosensor based on GNPs as a sensing platform to directly detect atrazine. GNPs were assembled on the gold electrode surface, which utilized the —SH group connecting bare gold electrode and GNPs through Au—S bond [29]. Then specific antibody was immobilized on the GNPs surface, and atrazine in sample can be directly detected. Various experimental parameters have been optimized for atrazine detection and selectivity, stability, and regeneration tests have been performed. The constructed atrazine immunosensor showed high sensitivity, accepted stability and reproducibility. Therefore, it could be applied to atrazine analysis of agriculture product.

2. Experimental

2.1. Materials and reagents

Anti-atrazine monoclonal antibody (Anti-atrazine) was obtained from IL Co. (USA). Anhydrous ethanol, hydrochloric acid, potassium ferricyanide (K_3[Fe-$(CN)_6$]), potassium ferrocyanide ($K_4[Fe(CN)_6]$), Tween 20, and bovine serum albumin (BSA) were obtained from Beijing Dingguo Biotechnology Co. (Beijing, China). Atrazine, 3-mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC), chloroauric acid ($HAuCl_4{\cdot}4H_2O$), fenvalerate, deltamethrin, dichlorodiphenyltrichloroethane (DDT), dimethoate, β-benzene hexachloride (β-BHC), n-hydroxysuccinimide (NHS), and mercaptoethylamine (MEA) were purchased from sigma (St. Louis, MO, USA). All chemicals and solvents were of commercially available analytical reagent grade, and ultrapure water was used throughout this work.

Anti-atrazine was dissolved with 0.01 M phosphate buffer solution (PBS, pH 7.4) and stored at 4 °C. Atrazine was dissolved with PBS containing 0.05% (v/v) Tween 20 (PBST). Blocking buffer solution was composed of PBS containing 1% (w/v) BSA.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS) measurements were carried out with a CHI 660D electrochemistry workstation (Shanghai CH Instrument Company, China). All experiments were performed using a conventional three-electrode system with a gold electrode (2.0 mm diameter) as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode in a 5-mL voltammetry cell at room temperature (25 °C). Transmission electron microscope (TEM) was carried out on JSM-6380 (JEOL). UV-Visible spectrum was recorded on UV-2450 ultraviolet-visible spectrophotometer (SHIMADU).

2.3. Synthesis of GNPs

According to the literature [29] with slight modification, GNPs were synthesized by the chemical reduction method. Firstly, all glassware were thoroughly cleaned in freshly prepared aqua regia (HCl/HNO_3, 3:1), rinsed with ultrapure water and dried prior to use. 3.0 mL 1% (w/v) sodium citrate solution was quickly added to 100 mL 0.01% (w/v) boiling $HAuCl_4{\cdot}4H_2O$ solution with magnetic stirring. Boiling continued for 8 min, then stop heating. Throughout the process, the solution color from yellow to gray in 2 min, then from gray to wine red and the mixture was stirred for 15 min until the color turned to bright red. The solution was cooled to the room temperature and kept at 4 °C.

2.4. Sample preparation

The fresh maize particles were put in oven for 12 h in 60 °C. The dried maize particles were smashed and sieved (20 meshes). Then maize sample (0.1 g) was extracted with PBST (10 mL) for 30 min using an automatic shaker. The crude extract was centrifuged at 12,000 rpm for 10 min. The supernatant was then diluted with PBST buffer. The sample was passed through a 0.45-μm filter before used.

2.5. Preparation of anti-atrazine/GNPs/gold electrode

The surface of the bare gold electrode was polished repeatedly with 0.1, 0.3, 0.05 μm alumina powder on microfiber cloth to obtain a mirror surface. Subsequently, it was thoroughly rinsed with ultrapure water and sonicated first in absolute ethanol and then in ultrapure water for 3–5 min to remove undesired adsorbed particles and dried in air. Then the electrode pretreatment were performed by twenty successive CV sweeps between −0.2 and +1.6 V (vs. SCE) at scan rate of 50 mV s^{-1} in the 0.5 M H_2SO_4. Finally, the gold electrode was washed with ultrapure water and dried under nitrogen.

The pretreated gold electrode was immersed into 10 mM MPA solution for 2 h and 0.4 mg/mL MEA solution for 1 h at room temperature, respectively. At the end of this period, the electrode was rinsed with ultrapure water to remove unbounded molecules on the electrode surface. Then 20 μL of the GNPs solution was dropped onto the gold electrode surface and incubated for 2 h at room temperature to form a GNPs layer. After that, the resulting electrode was placed in 10 mM MPA solution for 2 h and activated by EDC/NHS (0.4 M: 0.1 M) for 10 min after washing with ultrapure water. Following that, 20 μL of the 500 ng mL^{-1} anti-atrazine solution was cast on the surface of modified electrode for overnight at 4 °C, and anti-atrazine/GNPs/gold electrode was obtained. At last, non-specific atrazine binding sites were blocked with 1% (w/v) BSA in PBS for 30 min at room temperature and stored at 4 °C in PBS before use.

2.6. Experimental measurements

Electrochemical experiments were carried out at room temperature. Fig. 1 shows the design of the assay. 20 μL of different concentration of atrazine (from 5 pg/mL to 500 ng/mL) were sequentially dropped onto the surface of modified electrode to incubate for 30 min and obtained atrazine/BSA/anti-atrazine/GNPs/gold electrode. Then the electrode was washed with 0.01 M PBS (pH 7.4) for removing unbinding atrazine. Amperometric responses of the immunosensor were recorded in 3 mL of 0.1 M pH 5.0 PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. CV was recorded at a potential range from −0.2 to 0.6 V (vs. SCE) with scan rate of 50 mV s^{-1}. DPV was recorded at a potential from 0.6 to −0.2 V (vs. SCE) with scan rate of 50 mV s^{-1}, amplitude 0.05 V, pulse width 0.2 s. EIS was recorded at a potential 0.2 V, frequency from 0.1 to 10^5, amplitude 0.005 V.

3. Results and discussion

3.1. Characterization of the GNPs and immunosensor

The morphology of GNPs was characterized by TEM [29]. The result of TEM indicates that the average diameter of prepared GNPs