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Electrochemical immunoassay platform for high sensitivity detection of indole-3-acetic acid

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ABSTRACT

A label-free electrochemical immunosensor for sensitive detection of indole-3-acetic acid (IAA) was developed using gold nanoparticles (AuNPs) functionalized with horseradish peroxidase labeled immunoglobulins G (AuNPs-HRP-IgG) as signal amplification probe and rat monoclonal antibody against IAA as capture probe. AuNPs-HRP-IgG could be immobilized and through the interaction between 4-aminophenylboronic acid and a single N-glycosylation site, which exist in the CH2 domain of IgG. The electrochemical immunosensor was characterized by differential pulse voltammetry (DPV) in 0.1 M PBS (pH 7.4) containing $Fe(CN)_6^{3-/4-}$ as redox probe. The decreased oxidation peak current of $Fe(CN)_6^{3-/4-}$ was used to monitor the antibody–antigen interaction. The electrochemical immunosensor exhibited a linear range from 1×10^{-9} to 5×10^{-6} M with a limit of detection of 5.5×10^{-10} M (S/N = 3). The proposed electrochemical immunosensor could well discriminate IAA from other phytohormones, such as gibberellin, abscisic acid and salicylic acid. In addition, IAA extracted from leaf of mung bean sprouts was detected using the developed immunosensor for evaluating its applicability, and the recovery was from 93.5% to 106.75%.

1. Introduction

Phytohormones are important regulators produced by various plants, which play crucial role in the regulation of plant germination, growth, reproduction and cell division [1]. Indole-3-acetic acid (IAA) is one of the traditional phytohormone, which can regulate processes such as elongation and differentiation of cells. The content of IAA in most plant issues and seeds is very low (ng/g level) [2,3]. Therefore, the detection methods for IAA need high sensitivity. At present, conventional methods for IAA detection are capillary electrophoresis (limit of detection (LOD), 1.8×10^{-8} M) [3], high performance liquid chromatography (LOD, 5.71×10^{-6} M) [4], gas chromatography–mass spectrometry (LOD, 8.56×10^{-9} M) [5], liquid chromatography–mass spectrometry (LOD, 1.26×10^{-5} M) [6], solid-phase enzyme immunoassay (LOD, 4.0×10^{-10} M) [7] and electrochemical detection based on chemically modified electrodes (LOD, 1.5×10^{-7} and 2.0×10^{-8} M) [8,9]. It can be concluded that the electrochemical detection technique has comparable detection sensitivity with chromatography methods and capillary electrophoresis. However, chromatographies require sophisticated and expensive instrumentation, the time-consuming purification process, and skill-operator. Moreover, the purification process is extremely expensive and adverse to the environment and human health due to the use of large amounts of organic solvents [6]. In addition, the mass spectrometry-based methods needed high temperatures reached in the injector, which might cause the decomposition of the labile IAA. Solid-phase enzyme immunoassay showed high detection sensitivity and high selectivity for IAA. But it is time-consuming, expensive and multistep process. Therefore, a rapid, precise, low-cost and convenient assay method for detecting IAA in plants is needed.

Electrochemical immunoassays based on specific antigen–antibody recognition have attracted growing attentions in IAA detection due to the advantages of high sensitivity, good selectivity, low cost, low reagent consumption, short response time, simple operation, miniaturized and inexpensive instrument. Li et al. fabricated an immunosensors for IAA detection based on enzyme-linked competitive immunoreaction between free IAA and IAA labeled with HRP to bind on the anti-IAA IgG immobilized on the sol–gel–alginate–carbon composite electrode surface [10]. The linear range was from 2.85×10^{-5} to 2.85×10^{-3} M. However, the detection sensitivity needed further improved. Wu et al. developed an amperometric method for the determination of IAA based on the multi-wall carbon nanotubes film coated glassy carbon electrode [8]. The oxidation peak current of IAA was used to monitor the IAA concentration. The linear range was from 1×10^{-7} to 5×10^{-5} M and the detection limit is 2×10^{-8} M. Though the detection sensitivity was high, the direct oxidation signal of

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IAA was used for its detection. Therefore, this method might be interfered with other electroactive substances in plant.

Because the content of most biomolecules and proteins are low in vivo, the high detection sensitivity is needed. Many efforts have been made to improve the detection sensitivity using various signal amplification strategies utilizing nanomaterials, such as graphene [11,12], gold nanoparticles (AuNPs) [13,14], Fe_3O_4 [15,16], CuO [17], carbon nanotubes [18,19], etc. As a novel carbon material with carbon atoms parked in a two-dimensional honeycomb lattice, graphene can provide an effective platform in electrochemical sensors for increasing electrode surface and facilitating electron transfer of redox mediator because of their large interfacial surface area and excellent electronic conductivity [20,21]. Simultaneously, AuNPs have attracted more and more attentions due to its high specific surface area, good conductivity and biocompatibility [22,23]. Therefore, the combination of AuNPs and graphene as electrode support could exhibit good electrochemical performance.

The aim of this work is to develop a novel electrochemical immunosensor for IAA detection based on the specific interaction between IAA and its antibody. For improving the detection sensitivity, the horseradish peroxidase (HRP) labeled immunoglobulin G (IgG) functionalized AuNPs were prepared and used as the signal amplification unit, where IgG could well recognize and capture the anti-IAA antibody. Based on the immunoreaction between IAA and antibody, the electrochemical response of the redox probe would significantly decrease and the content of IAA could be detected according to the change of electrochemical response. The fabricated electrochemical immunosensor showed high sensitivity and selectivity.

2. Experimental

2.1. Reagents and materials

IAA, gibberellin (Gb), abscisic acid (ABA), salicylic acid (SA), potassium ferricyanide, potassium ferrocyanide and chloroauric acid ($HAuCl_4$) were purchased from Aladdin (Shanghai, China). Trisodium citrate was provided by KaiTong Chemical Reagent Co., Ltd. (Tianjin, China). PEG3350 was purchased from Solarbio (Beijing, China). EDC and NHS were supplied by Alfa Aesar. 4-Aminophenylboronic acid (APBA) was purchased from Mayareagent (Zhejiang, China). Rat monoclonal antibody against IAA (Ab) was obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (China). 11-Mercaptoundecanoic acid (MUA) was purchased from J&K Scientific Ltd. (Beijing, China). HRP-labeled goat anti-rat IgG was supplied by Sangon Biotechnology (Shanghai) Co., Ltd. (China). Graphene was obtained from Nanjing XFNano Material Tech Co., Ltd. (China). AuNPs were synthesized according to previous report [24] and the average diameter of AuNPs was about 13 nm according to the image of transmission electron microscope (figure not shown). Phosphate buffer solutions (PBS) were prepared by mixing the stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and the pH was adjusted by NaOH or HCl. The double distilled deionized water was autoclaved and used throughout the experiments.

2.2. Conjugation of AuNPs-HRP-IgG

The horseradish peroxidase labeled immunoglobulin G functionalized AuNPs (AuNPs-HRP-IgG) conjugates were prepared according to previous reports with some modifications [25–27]. Briefly, the pH of 400 μL gold colloids was initially adjusted to 9.0 using 0.1 M Na_2CO_3. Then, 100 μL of HRP-IgG (1 mg/mL) was added into the gold colloids. The reaction is mainly based on the interaction between $-NH_2$ and −SH in HRP-IgG and AuNPs [26,28]. The mixture was shaken gently for 5 min and then incubated overnight at 4 °C. After that, the mixture was centrifuged at 12,000 rpm for 30 min. The supernatant was removed and the precipitation was re-dispersed in 1 mL 10 mM PBS (pH 7.4). The centrifugation was repeated for three times. Following that, the precipitation was dispersed in 1 mL 0.1% PEG3350 to block any possible remaining active sites and avoid the nonspecific interaction. 20 min later, the solution was centrifuged at 12,000 rpm for 30 min. After the supernatant was removed, the sediment was washed with 10 mM PBS (pH 7.4) for three times and re-dispersed in 1 mL 10 mM PBS (pH 7.4). The obtained AuNPs-HRP-IgG conjugates were stored at 4 °C when not in use.

2.3. Preparation of electrochemical immunosensor

The glassy carbon electrode (GCE, d = 3 mm, Gaoss Union, China) was first polished to mirror-like surface with 0.03 μm alumina powder on micro-cloth pad, and then it was sonicated in double distilled deionized water, anhydrous ethanol and double distilled deionized water successively. After dried with nitrogen blowing, 10 μL graphene nanosheets (1 mg/mL) was dripped on the electrode surface and dried under infrared lamp. The obtained electrode (graphene/GCE) was rinsed with double distilled deionized water and dried with nitrogen blowing. Then, the electrode was immersed into 3 mM $HAuCl_4$ solution containing 0.1 M KNO_3, and the gold nanoparticles were electrochemically deposited on the electrode surface using amperometry i–t technique at −0.2 V for 200 s. Finally, the electrode was washed with double distilled deionized water and dried with nitrogen blowing (the obtained electrode is noted as AuNPs/graphene/GCE). For confirming that the gold particles modified on the electrode surface were nano-scale, the SEM image of AuNPs/graphene/GCE (Fig. 1) was recorded on Hitachi S4800 scanning electron microscopy (SEM) system.

Subsequently, 10 μL MUA (2.5 mM) was dripped on the electrode surface and incubated for 1 h. Then, the electrode was rinsed with double distilled deionized water to remove the unimmobilized MUA. After the —COOH was activated by the mixed solution of 50 mM EDC and 100 mM NHS for 1 h, 10 μL APBA (2.5 mM) was casted on the surface of AuNPs/graphene/GCE and incubated for another 1 h at room temperature. The remained activated carboxyl was blocked with 0.1 M ethanolamine. Then, the electrode was rinsed thoroughly with double distilled deionized water to remove the physically adsorbed material (the electrode was noted as APBA/AuNPs/graphene/GCE). Afterwards, 10 μL

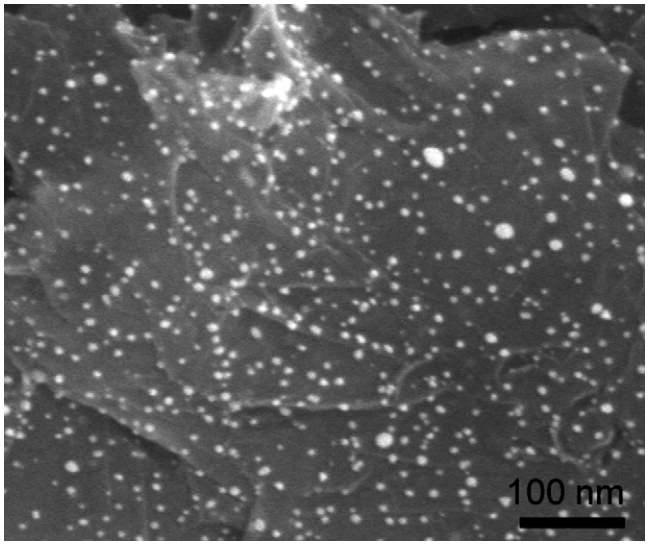

Fig. 1. SEM image of AuNPs/graphene/GCE.