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Short Communication

Competitive electrochemical immunosensor for the detection of histamine based on horseradish peroxidase initiated deposition of insulating film

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ABSTRACT

Competitive electrochemical immunosensor for the detection of histamine based on graphene modified electrode and horseradish peroxidase (HRP) initiated deposition of insulating film was developed. After the immobilization of anti-histamine antibodies onto graphene surface, free histamine and HRP tagged histamine molecules will compete to bind with the antibodies. The captured HRP could then catalyze the polymerization of 3,3′-dimethoxybenzidine (DB) in the presence of H_2O_2 to produce the deposition of an insulating polymer film, poly(3,3′-dimethoxybenzidine) (PDB) onto electrode surface. The deposited insulating PDB film resulted in the decrease of the electrochemical current of the electrode in $Fe(CN)_6^{3-/4-}$ solution, and the current change is proportional to the concentration of histamine detected. The proposed immunosensor displays wide linear range towards histamine detection (1 pg/mL–1 ng/mL) with a detection limit of 0.5 pg/mL. The immunosensor was applied to the detection of histamine in serum samples with satisfactory results.

1. Introduction

Histamine is a low molecular weight organic compound and an important biological marker in body fluid [1,2]. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in different tissues [3,4]. It is acted as a chemical mediator in inflammation, gastric acid secretion and neural modulation. So the sensitive and selective detection of histamine is of great importance in clinical diagnosis and allergy research for the study of allergic responses and a variety of pathological conditions [5–7].

Different techniques have been reported for the detection of histamine, such as enzyme based electrochemical biosensor utilizing various enzymes (e.g. histamine oxidase and histamine dehydrogenase) [8–10], immunosensors [11,12], high-performance liquid chromatography (HPLC) [13], liquid chromatography [14], and commercial enzyme immunoassays (EIAs) [15,16]. However, for enzyme based electrochemical biosensors, as there are many kinds of amines exist in the biological samples, these biosensors have the disadvantages of poor selectivity. The HPLC and liquid chromatography methods require complicated instruments. For EIAs, its sensitivity sometimes is not high enough for the detection of ultralow levels of histamine in the samples. On the contrary, compared to these techniques, electrochemical immunosensors has the advantages of high sensitivity, simple instrumentation, low cost and fast response [17–20].

In this work, we developed a competitive electrochemical immunosensor for the sensitive detection of histamine based on horseradish peroxidase (HRP) initiated deposition of insulating film [21]. Graphene was utilized to modify the electrode to increase the conductivity of the electrode and for the immobilization of antibodies. Anti-histamine antibodies were covalently immobilized onto graphene based on the carboxylic groups on the graphene. To carry out the competitive immunoassay, histamine was first labeled with enzyme HRP. When different concentrations of sample histamine were mixed with HRP labeled histamine (histamine-HRP), they will compete to bind with the antibodies on the electrode surface. The captured HRP on the electrode surface will catalyze the polymerization of 3,3′-dimethoxybenzidine (DB) in the presence of H_2O_2, leading to the formation of an insulating polymer film, poly(3,3′-dimethoxybenzidine) (PDB) onto the electrode surface [22,23]. Thus, the redox current of the electrode in $Fe(CN)_6^{3-/4-}$ solution was decreased, and the

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change of the redox current was proportional to the concentration of histamine detected.

2. Experimental

2.1. Reagents and apparatus

Rabbit anti-histamine antibody, horseradish peroxidase (HRP, Type VI), histamine and 3,3′-dimethoxybenzidine (DB) were purchased from Sigma–Aldrich. All other reagents were of analytical grade and deionized water (MilliQ, 18.2 MΩ cm) was used for preparing aqueous solutions throughout the experiments.

Electrochemical measurements were performed on a CHI-650D electrochemical workstation (Shanghai CH Instruments Co., China). A conventional three-electrode system was used with a glassy carbon (GC, 3 mm in diameter) as the working electrode, an Ag/AgCl electrode as the reference electrode and a platinum wire as the auxiliary electrode.

2.2. Synthesis of histamine-HRP

To conjugate HRP onto histamine, 100 μg histamine dissolved in 1 mL of phosphate buffer (pH 7.4) was initially reacted with 0.25% of glutaraldehyde for 30 min. Then 1 mg of HRP was added into the mixture and incubated for 4 h at room temperature. The removal of free histamine after conjugation was carried out by dialysis for 3 days with three changes of fresh buffer.

2.3. Immunosensor preparation

Anti-histamine antibody was immobilized onto graphene surface. Graphene was prepared from graphite oxide through a thermal exfoliation method according to our previous report [24,25]. To immobilize the antibodies onto graphene, typically, into 1 mL of graphene solution (2 mg/mL), EDC and NHS (100 mM) were added. The mixture was stirred for 4 h and centrifuged. Then, 1 mL of anti-histamine antibody solution (10 μg/mL) was added into the mixture. After another 12 h of reaction, the graphene solution was centrifuged and washed. The resulting graphene conjugates were stored at 4 °C in phosphate buffer solution before use.

For the modification of the electrode, 5 μL of the above prepared graphene conjugate solution was added onto the GC electrode surface. After dried, the electrode was then washed extensively with buffer and incubated in 1% BSA solution for 30 min to minimize non specific adsorption. Finally, 20 μL of different concentrations of histamine in buffer was mixed with 20 μL of histamine-HRP solution and then 10 μL of the mixture solution was added onto electrode and reaction for another 1 h. After extensive wash, the electrode was ready for measurement.

2.4. Electrochemical measurement

For electrochemical testing, the prepared immunosensor was incubated in solution containing 1.0 mM DB and 5.0 mM H_2O_2 for 30 min. After a brief washing with buffer, electrochemical testing was performed in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl.

3. Results and discussion

To modify the electrode with graphene, graphene was directly dropped onto the electrode surface. The good conductivity of graphene could increase the conductivity of the modified electrode. On the other hand, the large specific surface area of graphene could increase the loading of anti-histamine antibodies the increase the sensitivity of the immunosensor. The graphene modified electrode was first characterized in $Fe(CN)_6^{3-/4-}$ solution by cyclic voltammatry (CV). As shown in Fig. 1, compared to the bare electrode (curve a), the redox current of the graphene modified electrode was increased (curve b), indicating the modification of the electrode with graphene increased the conductivity of the electrode.

After the immobilization of anti-histamine antibodies onto graphene surface, the mixture containing different concentrations of free histamine and a specific concentration of histamine-HRP conjugate was applied onto the electrode surface. Free histamine and histamine-HRP conjugate will then compete to bind with the immobilized antibodies. As HRP could catalyze DB into insulate PDB to be deposited onto electrode surface, the redox current of the electrode will then be decreased. With the increasing of free histamine concentration, the histamine-HRP conjugate that captured onto the electrode will be decreased. Through such method, an "off–on" type electrochemical immunosensor was proposed for the detection of histamine. The immunosensor preparation and detection process was shown in Scheme 1.

The feasibility of the immunosensor for the detection of histamine was studied. Two immunosensors were prepared, one was for control experiment with no concentrations of free histamine detected, while the other was prepared for the detection of 1 ng/mL of histamine. Fig. 2 displays the CV responses of the two immunosensors after incubating in DB (1.0 mM) and H_2O_2 (5.0 mM) solutions [22]. It can be seen, regarding the immunosensor for control experiment, the redox current of the electrode was significantly suppressed (curve a). This was due to the fact that when no free histamine was present, the histamine that bound onto the electrode was entirely the histamine-HRP conjugate. So a substantial layer of insulating PDB film was produced because of the significant amount of HRP molecules captured onto the electrode surface. The resulted insulating PDB film prevented the electron transfer at the electrode surface, leading to the decrease of redox current. On the contrary, when 1 ng/mL of histamine was present in the sample, through competitive immuno-reaction, the amount of HRP captured onto electrode was decreased, so was the thickness of the PDB film. As a result, the redox current of the electrode was higher (curve b).

The immunosensor was also characterized by electrochemical impedance spectroscopy (EIS). Fig 3 represents the EIS results of the graphene-antibody conjugates modified electrode (curve a), the electrode prepared for the detection of 1 ng/mL of histamine (curve b) and the electrode after PDB film deposition (curve c). It

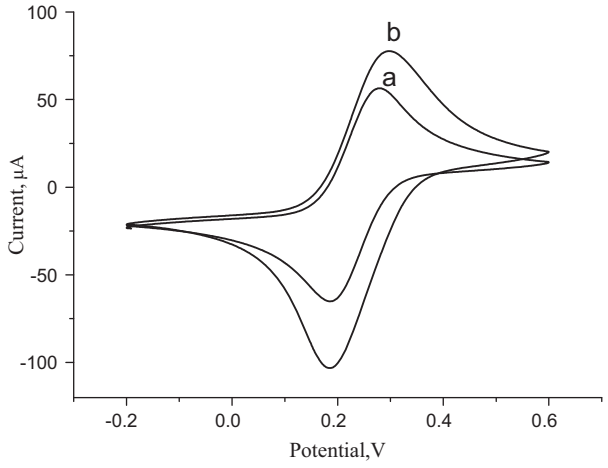

Fig. 1. Cyclic voltammatry responses of the bare (a) and graphene (b) modified electrodes in 5 mM $Fe(CN)_6^{3-/4-}$ solution. Scan rate: 0.1 V/s.