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A novel amperometric biosensor based on covalently attached multilayer assemblies of gold nanoparticles, diazo-resins and acetylcholinesterase for the detection of organophosphorus pesticides

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ABSTRACT

Using an ionic layer-by-layer self-assembly technique, colloidal gold nanoparticles (AuNPs) and diazo-resins (DAR) were immobilised on the surface of a p-aminobenzenesulfonic acid-modified glassy carbon electrode to form a matrix composite membrane for acetylcholinesterase (AChE) immobilisation. Photo-sensitive DAR was used as the assembly interlayer to convert the ionic bond into a covalent bond to improve the biosensor stability. These fabrication processes were followed by electrochemical impedance spectroscopy and cyclic voltammetry to verify the membrane formation. Because of the introduction of AuNPs/DAR/AChE biofilms, the modified electrode exhibited excellent electron transfer mediation and electrical conductivity. In addition, it exhibited high sensitivity in the range of linear concentration from 1.0×10^{-8} to 1.0×10^{-12} g L^{-1} with the detection limit of 5.12×10^{-13} and 5.85×10^{-13} g L^{-1} for malathion and methyl parathion, respectively. More importantly, the presented biosensor considerably improved stability because the electrostatic interaction was converted into covalent bonds by UV irradiation. It is a simple, cheap and stable method for quantitative detection of organophosphorus pesticides, and this method may pave a way for the sensitive, simple detection of different analytes without the need of expensive instrumentation.

1. Introduction

Pesticides are widely applied to control the quantity of weeds, fungi, insects and other harmful pests to improve the yield of crops [1,2]. Organophosphorus pesticides (OPs) are used worldwide because of their high efficiency as insecticides, wide range of control and low cost. However, OPs can also cause environmental pollution to water, air, agricultural products and soil, and therefore negatively affect human health. Irreversible OPs inhibit the activity of acetylcholinesterase (AChE) and impact to the function of the central nervous system, eventually leading to respiratory paralysis and death [3,4]. Therefore, a method for the easy, sensitive, and rapid detection of OPs is very important for both human health and environmental safety [5]. There are many techniques that can be used to OPs detection, including spectroscopy [6], gas or liquid chromatography [7–10], mass spectrometry [11], fluorescence biological method [12], and enzyme-linked immune system analysis [13]. Many of these methods are selective and accurate, but are time-consuming and require expensive instrumentation. To solve these problems, biosensors with rapid response, low cost, and high specificity and sensitivity have been developed since the twentieth century.

Electrochemical biosensors based on AChE are particularly attractive detectors because of their fast reaction and high sensitivity [14,15]. At the electrode surface, fixed AChE can catalyse the hydrolysis of acetylthiocholine chloride (ATCl), producing the electroactive product thiocholine (TCh); this shows an irreversible oxidation peak at about 0.68 V [16,17], which signifies pesticide detection. However, the oxidation peak has high oxidation potential, which is weak, resulting in poor sensitivity. Hence, a major research focus seeks to develop an electrochemical biosensor based on AChE for improving the performance of biosensors, therein reducing oxidation potential [18].

Carbon-based nanomaterials, colloidal gold nanoparticles (AuNPs) [19], nanostructured conductive polymers, or composites have been extensively investigated to increase the porosity, specific surface area and conductivity of the electrodes and to create platforms for enhanced loading of AChE, enabling significant increase in biosensors sensitivity [20]. AuNPs are a focus for researchers owing to their performance: they are highly conductive, have good biocompatibility [21], and have served as a key element in the synthesis and catalysis of organic reactions in biomedicine [21,22] and sensing applications [23,24].

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Furthermore, it has a lower oxidation potential and satisfactory selectivity compared to the other metals.

There have been many reports on various biosensors fabricated with AuNPs and AChE for the detection of OPs. A number of beneficial AChE immobilisation strategies have been developed based on the principles of encapsulation, physical adsorption and layer-by-layer self-assembly [25–29]. Among these methods, layer-by-layer self-assembly has been widely used because of its simplicity, low cost, wide selection of appropriate materials and precision of film composition control [30]. Layer-by-layer self-assembly by weak electrostatic force adsorption has been used to prepare enzyme biosensors in most previous studies; however, the stability of enzyme biosensors could be further improved through the strong covalent bond [31–33]. Thus, the purpose of this paper is to use layer-by-layer self-assembly technology to prepare stable covalently attached multilayer assemblies of P-ABSA/DAR/AuNPs/DAR/AChE (P-ABSA: *p*-acetamidobenzenesulphonyl azide) film in order to improve the stability of enzyme biosensors. Such a film would be an ideal material to detect OPs, having both a low detection limit and high sensitivity.

2. Experimental

2.1. Chemical reagents

Gold (III) chloride hydrate ($HAuCl_4$), Acetylcholinesterase (AChE 1000 U/mg) from electrophorus electrics (electric eel), Paraformaldehyde, Variamine Blue RT Salt and Acetylthiocholine chloride > 99% (ATCl) were purchased from Sigma Aldrich Phosphate buffered saline (PBS, pH 6.0) was prepared from KH_2PO_4, K_2HPO_4 and 0.1 M KCl. Sulfuric acid (H_2SO_4) and $ZnCl_2$ were purchased from Sinopharm Group Chemical Reagent Co., Ltd. P-amino benzene sulfonic acid was obtained from Aladdin reagent official website. In this study distillated water was used to obtain all solutions.

2.2. Apparatus

Electrochemical measurements were accomplished with a CHI660E (ShangHai Chenhua Co., China). The electrochemical cell consisted of a three-electrode system, the modified glassy carbon electrode (GCE) was used as the working electrode, Pt tablets acted as the counter electrode, and Hg/Hg_2Cl_2 electrode was applied as the reference electrode. All potentials in this paper were referenced to this saturated calomel electrode. The Portable UV-light WDH-204B (Shanghai Chi Tang Industrial Co., Ltd.) was applied to make the electrode Cross-linked. The decentralized and size of AuNPs was determined by transmission electron microscopy (TEM, Tecnai G2 S-twin of American company). UV–vis adsorption spectra were measured by Double-beam UV–vis spectrophotometer TU-1901 (Beijing Pu analysis of general instrument limited liability company).

2.3. Preparation of colloidal gold nanoparticles

Colloidal gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ in aqueous solution in this paper [34]. All glass-ware was cleaned in a bath by freshly prepared 1:3 HNO_3: HCl, then rinsed thoroughly in distilled water and dried in air. Next, 0.425 mL 0.01% $HAuCl_4$ in the 25 mL round bottom flask was boiled under vigorous stirring. The color of the solution was changed from light yellow to burgundy when 1.70 mL of 1% sodium citrate was joined to the solution. Boiling was continued for 15 min; the heating mantle was then removed, and stirring was continued for an additional 15 min. The resulting solution of colloidal gold nanoparticles were stored in a brown bottle and kept at 4 °C after it reached room temperature.

2.4. Preparation of diazo-resins

Measured 6.6 mL sulfuric acid was put to a 50 mL conical flask, which was in an ice bath. The following steps were to avoid light. When the temperature dropped to 0–5 °C, the 50 mL conical flask containing sulfuric acid was added Variamine Blue RT Salt (3 g, 10 mM) under the condition of stirring. Appropriate amount of polyoxymethylene (0.36 g, 12 tendency) were added to 50 mL conical flask at 0–5 °C constant stirring 3 h. $ZnCl_2$ (2 g) were dissolved in 12 mL distilled water (releasing heat), placed in 4 °C for later use. After the completion of the reaction, $ZnCl_2$ solution was added to the reaction system, then the mixed system were placed in the refrigerator overnight at 4 °C. In the second day, the mixed solution was filtrated till the sediment layer cracked but did not turn black. The product was dissolved in 60 mL of non-water ethanol, and 60 mL of ether was added to extract the solid. The solid was finally washed twice with 100 mL of ether. After repeating the above steps, the solid product of yellow diazo-resins was dried at Vacuum overnight and stored in the refrigerator.

2.5. Preparation of covalently attached multilayer assemblies of P-ABSA/DAR/AuNPs/DAR/AChE

Glassy carbon electrode was first polished with 0.05 μm alumina slurry and rinsed thoroughly with distilled water. Then the electrode was successively sonicated in acetone and distilled water. The cleaned GCE was analysed in 5 mM P-ABSA solution containing 0.1 M KCl at a scan rate of 10 mV/s over a potential range of + 0.5–+ 1.4 V (SCE). The GCE was functionalised with P-ABSA by electrochemical modification to obtain a sulfonic-fuctionalised monolayer film [35]. After the modification, the modified electrode was sonicated in water for 10 min to remove the physically adsorbed materials. P-ABSA was found to have modified the surface of the GCE with negatively charged sulfonate groups. The electrode was placed in a 5 mL containing 0.3 mg/mL DAR solution soak for 15 min, the positively charged diazo groups of the DAR solution were adsorbed onto the electrode surface by electrostatic attraction interactions. Then the modified electrode was immersed in the AuNPs solution for 15 min. As DAR is positively charged, it would be adsorbed to negatively charged AuNPs by electrostatic layer self-assembly, thus preparing the GCE/P-ABSA/DAR/AuNPs modified electrode. After this, the modified electrode was immersed in the DAR solution for 15 min 5 μL containing 5 U of AChE solution coated with the modified electrode surface was stood for 30 min. AChE was dissolved in a pH 7.5 PBS solution; because the isoelectric point of AChE is 5.6 (pI = 5.6) with negative charge, it was easy to combine the positively charged DAR to form GCE/P-ABSA/DAR/AuNPs/DAR/AChE modified electrode by electrostatic interaction. Finally, the fabricated films were exposed to UV light for a given time to ensure completion of the reaction. The principle of covalent bond formation is as follows. It is well known that the diazonium group is very active and can decompose rapidly under UV irradiation or heating. The DAR used in this paper contains such diazonium groups. Cationic phenyl groups can be formed in polyion chains, which can react easily with nucleophilic compounds. Here, the sulfonate groups on the electrode surface, modified by P-ABSA, are nucleophilic. It can be inferred that sulfonate groups at the electrode surface react readily with the diazonium groups of DAR under UV irradiation. Moreover, the carboxylate groups on AuNPs surfaces are nucleophilic, and thus it is plausible that there is a photoreaction between the carboxylate groups on the AuNPs surfaces and the diazonium group of DAR. Similarly, the carboxylate groups on AChE can also react with the diazonium group of DAR under UV irradiation. In this way, the covalently attached multilayer films were obtained. The deposition process of the films was conducted in the dark to avoid the decomposition of DAR. Scheme 1 shows the processes of layer-by-layer self-assembly of covalently attached multilayer films on the GCE.