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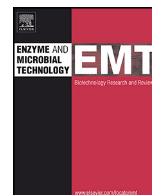

An acetylcholinesterase biosensor based on graphene–gold nanocomposite and calcined layered double hydroxide

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ABSTRACT

In this study, a novel acetylcholinesterase-based biosensor was fabricated. Acetylcholinesterase (AChE) was immobilized onto a glassy carbon electrode (GCE) with the aid of Cu–Mg–Al calcined layered double hydroxide (CLDH). CLDH can provide a bigger effective surface area for AChE loading, which could improve the precision and stability of AChE biosensor. However, the poor electroconductibility of CLDHs could lead to the low sensitivity of AChE biosensor. In order to effectively compensate the disadvantages of CLDHs, graphene–gold nanocomposites were used for improving the electron transfer rate. Thus, the graphene–gold nanocomposite (GN-AuNPs) was firstly modified onto the GCE, and then the prepared CLDH-AChE composite was immobilized onto the modified GCE to construct a sensitive AChE biosensor for pesticides detection. Relevant parameters were studied in detail and optimized, including the pH of the acetylthiocholine chloride (ATCl) solution, the amount of AChE immobilized on the biosensor and the inhibition time governing the analytical performance of the biosensor. The biosensor detected chlorpyrifos at concentrations ranging from 0.05 to 150 μg/L. The detection limit for chlorpyrifos was 0.05 μg/L.

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1. Introduction

Pesticide residues in food, livestock and water pose severe threat to human health [1]. Therefore, rapid determination and reliable quantification of pesticide compounds have become increasingly important for public security and health protection [2,3]. Biosensor is an analytical device incorporating a biological material with a suitable transducer that converts the biochemical signal into quantifiable electric signals [4]. Enzyme-based amperometric biosensors are important tools to detect pesticides in healthcare measure, food industry and environmental analysis [5,6]. These devices are designed to complement or replace the existing reference analytical methods such as gas/liquid chromatographic and mass spectrometric by simplifying or eliminating sample preparation, thus decreasing the analysis time and cost [7].

Metal nanoparticles are considered to be one kind of attractive nanomaterials due to their extraordinary advantages which include stability, conductivity, biocompatibility, low cytotoxicity and catalytic property [8,9]. With the rapid development of nanotechnology, various nanomaterials have been synthesized, which open new way to amplify the signal of biosensor [10]. Graphene nanosheets (GNs), a perfect two-dimensional (2D) carbon nanophase material found in 2004, have attracted tremendous attention [11,12], due to their exceptional thermal and mechanical properties, good chemical stability, high surface areas (calculated value, 2630 m^2/g), and excellent electrical conductivity [13,14]. Wang et al. have reported a biosensor based on acetylcholinesterase (AChE) immobilized on CdS-decorated graphene nanocomposite [15]. Li et al. have reported a sensitive amperometric biosensor through immobilizing AChE on porous-reduced graphene oxide [16]. Here, we prepared a very stable graphene–gold nanocomposite as modification material to fabricate an amperometric biosensor for pesticides detection.

Searching for a simple and reliable scheme to immobilize enzyme is of great importance. Layered double hydroxides (LDHs), also known as the anionic clays or hydrotalcite clays, can be expressed with the following general formula: $[M^{II}_{1-x}M^{III}_{x}(OH)_2] \times [A^{n-}_{x/n} \times mH_2O]$ [17]. Where, M^{II}_{1-x} are divalent cations (Mg^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}); M^{III}_{x} are trivalent cations (Al^{3+}, Cr^{3+}, Fe^{3+}), and A^{n-} is an interlayer anion (Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-}) compensating for the charge on the layers [18,19]. LDHs are an important class of host-guest materials consisting of positively charged metal

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hydroxide sheets with charge-balancing intercalated anions and water molecules [20,21]. By heating LDHs above 400 °C, the interlayer CO_3^{2-} can be removed. Therefore, the resulting calcination products of LDHs (CLDHs) can be used for removing inorganic anions by adsorption on the external surface of the layers, intercalation process or reconstruction behavior [22]. Nowadays, CLDHs have been paid more attention owing to their larger surface areas, higher metal dispersion, smaller crystallite size, better stability against sintering, higher thermal stability, better dispersion of the active species, and less diffusion resistance than LDHs [23]. In previous works, LDHs have been demonstrated as attractive enzyme immobilization matrix [24,25]. To date, however, the application of CLDHs in AChE biosensors has been still very less reported.

However, the electroconductibility of CLDHs is not very good, which can lead to the low sensitivity of enzyme biosensor. The graphene–gold nanocomposites which have excellent conductivity and biocompatibility, could effectively compensate the disadvantages of CLDHs, and then improve the performance of the biosensor.

In this study, we described the application of CLDHs as enzyme immobilization matrix to construct highly performance-enhanced AChE biosensor. CLDH can result an increase of effective surface area for AChE loading, which could improve the precision and stability of the AChE biosensor. Graphene–gold nanocomposite was dropped on the surface of the glassy carbon electrode, which obviously improved the conductivity of the modified electrode. Compared with other kinds of electrochemical AChE biosensors, it was much better in sensitivity, reproducibility and stability for the determination of pesticide, and it could be applied in real samples measurement.

2. Experimental

2.1. Apparatus

Electrochemical measurements were performed with CHI660D electrochemical workstation (Shanghai Chenhua Co., China). The working electrode was glassy carbon electrode (GCE) (d = 3 mm) or modified GCE. A saturated calomel electrode (SCE) and platinum wire electrode were used as reference electrode and auxiliary electrodes, respectively. Scanning electron micrographs (SEM) was studied by Sirion 200 SEM.

2.2. Reagents and materials

Acetylcholinesterase (Type C3389, 500 U/mg from electric eel), acetylthiocholine chloride (ATCl), chlorpyrifos were purchased from Sigma (USA). $HAuCl_4$ was obtained from National Chemical Pharmaceutical Co., China. Graphene was obtained from nanoon Co., China. The 0.1 M pH 7.5 phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. Cu $(NO_3)_2{\cdot}3H_2O$, Mg $(NO_3)_2{\cdot}6H_2O$, Al $(NO_3)_3{\cdot}9H_2O$ and other reagents were of analytical grade. All solutions were prepared using double distilled water.

2.3. Synthesis graphene–gold nanocomposites

2.0 mg graphene was added into 105 μL of 0.01 M $HAuCl_4{\cdot}4H_2O$ by sonicating until GN disperse equably. Then 105 μL of 0.01 M sodium citrate, 10.0 mL ethanol and 20.0 mL deionized water were added to the above suspension in sequence. 125 μL of 0.05 M $NaBH_4$ ice-cold solution were added to the above mixture and stirred until the color of the solution did not change. After stirred for an additional 10 h, the suspending liquid was separated by centrifuging at a speed of 16,000 rpm, washed with deionized water for several cycles. Finally, the precipitates were redispersed in 5 mL pH 7.5 PBS, and then stored in a brown bottle at 4 °C for use [26,27].

2.4. Preparation of Cu–Mg–Al CLDH

The preparing method for Cu–Mg–Al LDH was similar to what reported in literature [23,28]. In brief, 20 mL solution containing 1.208 g of $Cu(NO_3)_2{\cdot}3H_2O$, 3.846 g $Mg(NO_3)_2{\cdot}6H_2O$ and 3.751 g $Al(NO_3)_3{\cdot}9H_2O$ was titrated with 20 mL mixture solution of 2.40 g NaOH and 5.30 g Na_2CO_3 under vigorous stirring. During the synthesis, the temperature was maintained at 25 °C. The resulting suspension was then maintained at 65 °C for 1 h with stirring. The obtained product was filtered and washed thoroughly with deionized water until a neutral pH was observed, then dried at 60 °C for 2 days in air. Thus, the Cu–Mg–Al LDH was obtained. The Cu–Mg–Al CLDH was prepared by heating Cu–Mg–Al LDH in a muffle furnace at 500 °C for 7 h.

2.5. Preparation of CLDH-AChE composite

The colloidal suspension of CLDH (2 mg/mL) was prepared by dispersing CLDH in deionized water stirring overnight. Then, a stock solution of AChE was mixed with the colloidal solution of LDHs (2 mg/mL) with volume ratio 1:1 to obtain the suspension of CLDH-AChE. The resulting suspension was stored at 4 °C for use.

2.6. Preparation of CLDH-AChE/GN-AuNPs/GCE biosensor

A GCE was polished carefully to a mirrorlike surface with 0.3 μm and 0.05 μm Al_2O_3 paste and washed using sonication with ethanol, nitric acid and doubly distilled water. Before the modification of the electrode, a potential scan was applied from −0.6 to 1.0 V in 0.5 mol/L H_2SO_4 for 300 s until a steady-state curve was obtained. 5 μL GN-AuNPs solution was coated onto the pretreated GCE and dried in the air. The obtained GN-AuNPs/GCE was washed thoroughly with double distilled water. Then a 5.0 μL CLDH-AChE solution (100 mU) was dropped onto the GCE and dried in air at room temperature. After washing carefully with pH 7.5 phosphate buffer solutions, the CLDH-AChE/GN-AuNPs/GCE was obtained. The CLDH-AChE/GN-AuNPs/GCE was stored at 4 °C when not in use. The scheme of the preparation of CLDH-AChE/GN-AuNPs/GCE biosensor was shown in Fig. 1.

2.7. Electrochemical detection of pesticides

The CLDH-AChE/GN-AuNPs/GCE biosensor was employed for the determination of pesticide by differential pulse voltammetry (DPV) method. The performance of the biosensor was investigated by its DPV response in pH 7.5 PBS solution containing 1.0 mM ATCl. Then the electrode was rinsed with water and incubated in an aqueous solution containing a certain concentration of chlorpyrifos for 10 min. Finally, it was transferred into the 1.0 mM ATCl solution for DPV measurements at the same condition. The inhibition rate of pesticides was calculated as follows:

$$\text{inhibition } (\%) = \frac{\left(I_{\text{P,control}} - I_{\text{P,exp}}\right)}{I_{\text{P,control}}} \times 100\% \qquad (1)$$

where, $I_{\text{P,control}}$ was the peak current of ATCl on CLDH-AChE/GN-AuNPs/GCE with pesticides inhibition, $I_{\text{P,exp}}$ was the peak current of ATCl on CLDH-AChE/GN-AuNPs/GCE with pesticides inhibition. Inhibition (%) was plotted against the concentrations of the pesticides to obtain linear calibration graphs.

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