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Amperometric detection of triazophos pesticide using acetylcholinesterase biosensor based on multiwall carbon nanotube–chitosan matrix

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Abstract

A simple method for immobilization of acetylcholinesterase (AChE) on multiwall carbon nanotubes (MWNTs)–chitosan (MC) composite was proposed and thus a sensitive, fast and stable amperometric sensor for quantitative determination of organophosphorous insecticide was developed. Atomic force microscopy showed that this matrix possessed homogeneously netlike structure, which prevented enzyme from leaving out of the electrode. MWNTs promoted electron transfer reactions at a lower potential and catalyzed the electro-oxidation of thiocholine, thus increasing detection sensitivity. Based on the inhibition of organophosphorous insecticide to the enzymatic activity of AChE, using triazophos as a model compound, the conditions for detection of the insecticide were explored. Under optimal conditions, the inhibition of triazophos was proportional to its concentration in two ranges, from 0.03 to 7.8 μM and 7.8 to 32 μM with a detection limit of 0.01 μM. A 95% reactivation of the inhibited AChE could be regenerated for using pralidoxime iodide within 8 min. The constructed biosensor processing prominent characteristics and performance such as good precision and reproducibility, acceptable stability and accuracy, fast response and low detection limit has potential application in the characterization of enzyme inhibitors and detection of toxic compounds against to enzyme.

Keywords: Acetylcholinesterase; Biosensor; Chitosan; Multiwall carbon nanotube; Triazophos

1. Introduction

Organophosphates (OP) compounds, as one group of the most commonly applied pesticides in agriculture, are typical examples exhibiting fairly high toxicity. For these reasons, rapid determination and reliable quantification of trace level of OP compounds are significant to healthiness and environment [1]. The toxic action of OP compounds is due to their ability to irreversibly modify the catalytic serine residue in acetylcholinesterases (AChE) and subsequent inhibition of the AChE effectively prevents nerve transmission by blocking breakdown of the transmitter choline [2]. Biosensors based on the inhibition to AChE have been widely used for the detection of OP and carbamates pesticides. When AChE was immobilized on the working electrode surface, its interaction with the substrate of acetylthiocholine obtained an electro-active product of thiocholine, which produced an irreversible oxidation peak. The inhibition of OP on AChE was monitored by measuring the decline of oxidation current of thiocholine.

Comparing with other analytical techniques such as gas and liquid chromatography [3–5], enzyme based electrochemical biosensors represent good selectivity, sensitivity, rapid response, and miniature size for determination of pesticide. Among the fabrication of biosensor, immobilization of enzyme to solid electrode surface is a crucial step for the design of the biosensor [6]. A key requirement of enzyme immobilization is attachment without the bioactivity being sacrificed [7].

Chitosan (CS) contains large groups of $–NH_2$ and –OH has been widely used as a modifying reagent to prepare modified electrode due to its excellent biocompatibility, nontoxicity, cheapness, easy-handling and high mechanical strength [8]. It is preferable to maintain the high biological activity of the immo-

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bilized biomolecules and enhance the sensitivity of the sensor. Because of the advantages in sensing field including small size with larger surface, easy immobilization of protein with retention of activity [9], particularly the ability to facilitate electron transfer when being used as electrode [10,11], carbon nanotubes (CNTs) are extremely promising for preparation of amperometric biosensors and applications in biochemical sensing domain [12,13].

This work proposed a simple method for immobilization of AChE by using glutaraldehyde as cross-linker to multiwall carbon nanotubes (MWNTs)–chitosan (MC) composite, leading to a stable AChE biosensor for rapid determination of triazophos, a model OP compound, quantitatively. The presence of MWNTs reduced the working potential by catalyzing the electrochemical oxidation of enzymatically formed thiocholine. Compared with other kinds of electrochemical AChE biosensor design, this method was simple, rapid and more sensitive for pesticide determination with much lower detection limit [14,15]. The proposed sensor showed acceptable stability and sensitivity, which had potential application in AChE-inhibitors (OP or carbamates pesticides) analysis and environmental monitoring.

2. Experimental

2.1. Reagents

Acetylthiocholine chloride (ATCl), acetylcholinesterase (Type C3389, 500 U/mg from electric eel) and glutaraldehyde (25%) were purchased from Sigma–Aldrich (St. Louis, USA) and used as received. Triazophos was obtained from AccuStandard (USA). Multiwall carbon nanotube was a gift from the Institute of Nanometer, Central China Normal University. Chitosan (95% deacetylation), bovine serum albumin (BSA), phosphate buffer solution (PBS, pH 7.0) and other reagents used were of analytical reagent grade. All solutions were prepared using double distilled water.

2.2. Apparatus

Electrochemical measurements were performed on a Bioanalytical System (BAS, cv-50w, USA) with a conventional three-electrode system comprising platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference and AChE immobilized on MWNTs-CS (AChE-MC) modified glass carbon electrode (AChE-MC/GCE) as working electrode. Atomic force microscopy (AFM) experiments were performed on SPA-300 HV atomic force microscopy with a SPI 3800 controller (Seiko, Japan).

2.3. Preparation of AChE biosensor

GCE was polished to mirror finish using the BAS-polishing kit with 0.3 and 0.05 μm Al_2O_3 paste. After sonicated with ethanol and water, the electrode was applied a potential of +1.75 V under string in pH 5.0 PBS for 300 s and then was scanned from +0.3 to +1.25 V and +0.3 to −1.3 V until a steady-state curve was obtained [16] activation of the GCE involved formation of a new phase containing a substantial amount of microcrystallinity and graphite oxide, thus increasing the hydrophilicity of the surface [17]. The AChE biosensor was prepared according to our previous work [16]. Briefly, 2.0 μL of mixture of MWNTs, chitosan and glutaraldehyde were coated on a pretreated GCE with final contents of 0.12% (w/v), 0.48% (w/v), 0.47% (v/v), respectively, and allowed for reaction at 20 °C for 4 h. After being washed thoroughly with double distilled water, the electrode was coated with 4.0 μL AChE solution. The obtained biosensor was stored at 4 °C when not in use.

2.4. Electrochemical detection of pesticide

For the measurement of triazophos, the obtained AChE-MC/GCE was first immersed in PBS solution containing different concentrations of standard triazophos solution for 10 min, and then transferred to the electrochemical cell of 1.0 mL pH 7.0 PBS containing 0.4 mM ATCl to study the electrochemical response by cyclic voltammetry (CV) between +0.1 and +1.0 V (versus SCE). The inhibition of pesticide was calculated as follows:

$$\text{Inhibition}\,(\%) = \frac{i_{\text{P,control}} - i_{\text{P,exp}}}{i_{\text{P,control}}} \times 100\%$$

where $i_{\text{P,control}}$ was the peak current of ATCl on AChE-MC/GCE, $i_{\text{P,exp}}$ was the peak current of ATCl on AChE-MC/GCE with triazophos inhibition. Inhibition (%) was plotted against the concentrations of the triazophos to obtain a linear calibration graph.

2.5. Enzyme reactivation

After AChE-MC/GCE was exposed to pesticide, it was washed with PBS and reactivated with 4.0 mM pralidoxime iodide for 8 min, then transferred to electrochemical cell of 1.0 mL pH 7.0 PBS containing 0.4 mM ATCl to study the electrochemical response. The reactivation efficiency (R, %) was estimated as follows:

$$R\,(\%) = \left(\frac{i_{\text{r}} - i_{\text{P,exp}}}{i_{\text{P,control}} - i_{\text{P,exp}}}\right) \times 100\%$$

where i_{r} was the peak current of ATCl on AChE-MC/GCE with pralidoxime iodide reactivation.

3. Results and discussion

3.1. Electrochemical behavior of AChE-MC/GCE

Fig. 1 showed the cyclic voltammograms of various electrodes in absence and presence of 0.4 mM ATCl in pH 7.0 PBS. No peak was observed at GCE (curve a) and AChE-MC/GCE (curve b) in PBS. When 0.4 mM ATCl was added into PBS, the cyclic voltammograms of AChE-MC/GCE showed an irreversible oxidation peak at 660 mV (curve d). Obviously this peak came from the oxidation of thiocholine, hydrolysis product of ATCl, catalyzed by immobilized AChE. Furthermore, this peak current was much higher and the peak potential shifted neg-