

Acetylcholinesterase biosensor based on chitosan/ZnO nanocomposites modified electrode for amperometric detection of pesticides

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Abstract. A novel photoelectrochemical biosensor incorporating nanosized ZnO semiconductor crystals with enzyme to enhance photochemical reaction has been investigated. In this work, the ZnO nanoparticles and acetylcholinesterase (AChE) were immobilized on Pt electrode by chitosan (CHI) via layer-by-layer technique (LbL) to fabricate a biological-inorganic hybrid system. Micrographs of (ZnO/CHI/AChE) films were obtained by scanning electron microscope, and photoelectrochemical properties of the resulting biosensors were measured by a three electrodes system and an ultraviolet lamp. Under ultraviolet light, the photo-effect of the ZnO nanoparticles showed enhancement of the biosensor to detect pesticide. Based on the inhibition of organophosphate pesticides on the AChE activity, using malathion as a model compound, the inhibition of malathion was proportional to its concentration ranging from 0.25 to 1.50 and from 1.75 to 10.00 μM , with a detection limit of 10 nM estimated at a signal-to-noise ratio of 3. The developed biosensor exhibited good reproducibility, thus providing a new promising tool for analysis of enzyme inhibitors.

1 Introduction

Recent years, biosensor techniques have made encouraging progress and draw people's wide concern due to the advantages of low cost, easy operation, fast response and high sensitivity [1]. AChE is an enzyme which stabilizes the levels of the neurotransmitter acetylcholine by catalyzing the hydrolysis of acetylcholine to thiocholine. The catalytic activity of AChE is inhibited by trace amounts of organophosphorus and carbamate pesticides. Biosensors based on the inhibition of AChE are most frequently used for both identification and quantification of pesticides [2]. This method has emerged as a promising technique providing a rapid response and signaling for toxicity analysis, environmental monitoring, food quality control, and military investigations. However, the efficient electrical communication between enzyme's redox center and solid electrode surfaces still forms a barrier [3], due to the fact that the enzyme active site is deeply buried in the proteins shell. Therefore, it is important to find a feasible way to further enhance the efficiency of the electrical communication. In recent years, zinc oxide, one of the most attractive functional semiconductor materials, has gained significant attention and emerged as a class of promising materials widely used in photodetectors, gas sensors, solar cells, transparent conductors, and short-wavelength optoelectronic devices or catalysts [4]. In this report, we describe the fabrication, characterization and analytical performance of AChE biosensor based on ZnO nanoparticles. Our experiment shows that these ZnO nanoparticles can significantly enhance the current sensitivity of AChE enzyme electrode. In the photoelectrochemical system, the photovoltaic effect of the ZnO nanoparticles can play an interesting role in the catalytic ability of AChE and further improve the sensitivity of AChE biosensor. This fabrication method of AChE biosensor is simple and effective, which can be potentially applied in other enzyme/semiconductor system.

2 Experimental

2.1 Materials

Acetylcholinesterase (AChE), acetylthiocholine chloride (ATCl), phosphate buffered saline containing 0.1M KCl (PBS, 0.01 M, pH 7.4), zinc chloride, dithiobis (2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA) and chitosan were purchased from Sigma Aldrich. All aqueous solutions were prepared with doubly distilled water. PBS buffer was employed as supporting electrolyte. All experiments were performed in PBS at room temperature, approximately 25 °C.

2.2 Preparation of ZnO nanoparticles

ZnO nanoparticles were synthesized by precipitation from 2-propanol [4].

2.3 Fabrication of the biosensor

The chitosan-coated Pt electrode was immersed in a ZnO solution (2 mg/mL, pH 3.8, treated with acetic acid) for 15 min at room temperature to deposit the first monolayer of ZnO on the electrode surface, and washed with the PBS for about 5 min. The ZnO-modified electrode was then immersed in polycation chitosan for 15 min and rinsed in PBS for 5 min. The CHI-ZnO-modified electrode was then immersed in the AChE solution (100 U/mL, 0.1% BSA) for 20 min to immobilize AChE film, and also washed with the phosphate buffer solution for about 5 min. In order to make the ZnO being distributed on the electrode uniformly, the ZnO solution was stirred by a magnetic chip. After then, the electrode was removed from phosphate buffer solution, rinsed gently with purified water, blotted dry around the edges and covered to protect from dust.

2.4 Apparatus and measurements

Cyclic voltammetric and amperometric measurements were used to characterize the (ZnO/CHI/AChE) biosensor. For amperometric measurements, the electrochemical cell was stirred with a small magnetic bar at 150 rpm. After stabilization of the capacitive current, the enzymatic reaction was initiated by addition of 3mM ATCl substrate and the response of the sensor was measured.

Inhibition measurements were carried out in a two steps batch procedure [1] by measuring the response of the sensor to additions of a constant amount of ATCl substrate before and after inhibition with pesticides. All inhibition measurements were carried out with malathion as a model organophosphate pesticide. The electrodes were incubated for 10 min in doubly distilled water in the absence and presence of concentrations of malathion ranging from 10^{-8} - 10^{-5} M. After the (ZnO/CHI/AChE)/Pt was inhibited by malathion, it was washed with PBS and reactivated with 4.0mM pralidoxime iodide for 8min, then transferred to electrochemical cell of 1.0mL pH 7.0 PBS containing 0.3mM ATCl to study the electrochemical response. The reactivation efficiency ($R\%$) was estimated as the method adopted earlier[5].

3 Results and Discussion

3.1 Characterization of (ZnO/CHI/AChE) film

Fig. 1A shows the typical scanning electron microscope (SEM) of the ZnO nanoparticles, which indicates that the sample is composed of a large quantity of well-dispersed spherical nanoparticles. The surface of every particle is rough and with many smaller particles. It appears that these primary small nanoparticles interconnected with one another to form larger secondary particles with recognizable boundaries or voids between the component subunits. When AChE was coated on the ZnO nanoparticles, the obtained composite was much thicker than the raw ZnO (Fig. 1B).

3.2 Photo-enhanced effect of (ZnO/CHI/AChE) modified electrode

Fig. 2A shows the enzymatic photo-activity of (ZnO/CHI/AChE) modified electrode in the dark and under ultraviolet. In the dark an anode response current can be observed for (ZnO/CHI/AChE) modified electrode. Under ultraviolet, the response current is larger than that in the dark. Fig. 2B shows that the current response under ultraviolet is twice as much as that in the dark. The biosensor achieved 95% of the steady state current with 4 s. This is a good symbol for the biosensor. The

larger response current means higher sensitivity than biosensors which do not use the photoelectron effect, implying that the ZnO nanoparticles possess a photo-enhanced effect for the (ZnO/CHI/AChE) modified electrode. Under ultraviolet, the electron and hole were generated, respectively in the conduction and valence bands.

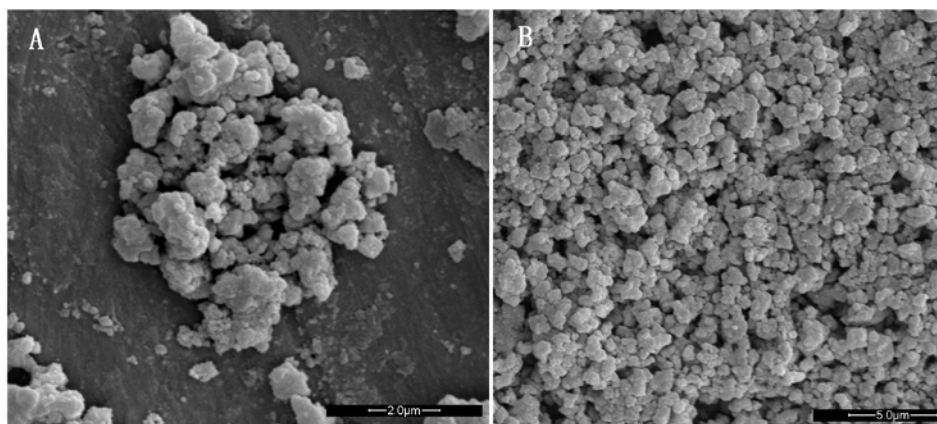


Fig. 1 Typical SEM images of ZnO nanoparticles (A) and the (ZnO/CHI/AChE) composite (B)

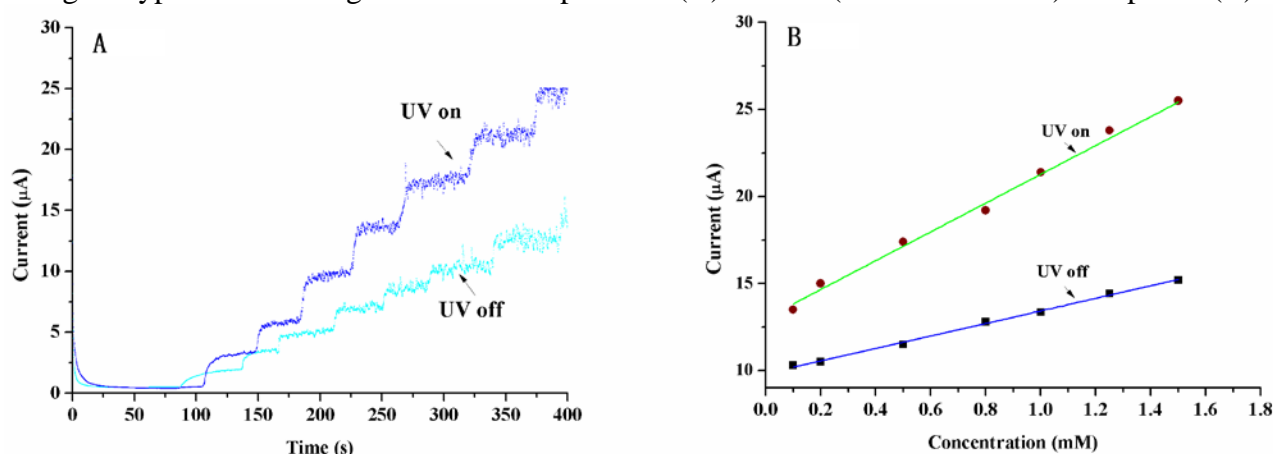


Fig. 2 (A) Amperometric response of (ZnO/CHI/AChE)/Pt electrode upon subsequent addition of 0.3 mM ATCl to 10 mL of 0.1 M PBS (pH 6.8) at 0.6V: (UV on) illumination, (UV off) dark. (B) Calibration curve for the measurements under illumination and dark, respectively.

3.3 Electrochemical Performance of (ZnO/CHI/AChE)/Pt electrode

Cyclic voltammetry (CV) was employed to investigate the (ZnO/CHI/AChE)/Pt electrode over a potential range from -0.2 V to 0.8 V in 0.1 M PBS buffer (pH 6.8) at 0.05 Vs⁻¹.

Fig. 3A shows the CV behaviors of different modified electrodes in 0.1 M PBS. No peak was observed when the bare Pt electrode (curve a) and (ZnO/CHI/AChE) (curve b) modified Pt electrode were placed in PBS (pH 6.8). In 0.3 mM ATCl PBS, the CV response at the (ZnO/CHI/AChE)₅/Pt electrode displayed an irreversible oxidation peak at 0.68 V (curve c, d and e). Obviously this peak arose from the oxidation of thiocholine, which was the hydrolysis product of ATCl and catalyzed by the immobilized AChE. As shown in Fig. 3B, the inhibition efficiency of malathion was a linear function of its concentration from 0.25 to 1.5 and 1.75 to 10.00 μM. The linearization equation were $I\% = 23.50c + 12.56\%$ and $I\% = 3.65c + 48.65\%$, with the correlation coefficients of 0.9972 and 0.9970, respectively. The detection limit was calculated to be 10 nM at a signal-to-noise ratio of 3.

3.4 Reactivation of the biosensor

The (ZnO/CHI/AChE)/Pt electrode inhibited by malathion can be completely reactivated when using nucleophilic compounds such as pralidoxime iodide. With increasing reactivation time, the reactivation efficiency increased and reached a constant value after 8 min. The inhibited AChE could be regenerated more than 92.5% of its original activity after immersing in 3.0 mM pralidoxime iodide. Based on this reactivation procedure, the proposed biosensor can be used repeatedly with an acceptable reproducibility.

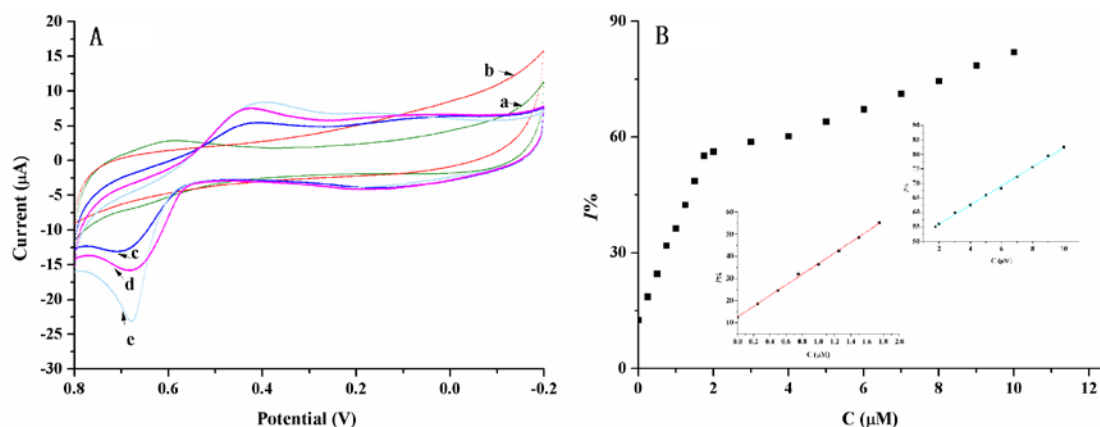


Fig. 3 (A) Cyclic voltammograms of bare Pt electrode (a), (ZnO/CHI/AChE)/Pt electrode (b) in pH 6.8 PBS, (ZnO/CHI/AChE)/Pt electrode in pH 6.8 PBS containing 0.3 mM ATCl, and after inhibition by malathion of 10^{-6} M (c), (ZnO/CHI/AChE)/Pt electrode in pH 6.8 PBS containing 0.3 mM ATCl, UV off (d), (ZnO/CHI/AChE)/Pt electrode in pH 6.8 PBS containing 0.3 mM ATCl, UV on (e). (B) Relationship between peak currents and malathion concentrations. Insets show the calibration curves for malathion determination.

4 Conclusion

We have demonstrated a simple and effective method for the fabrication of enzyme biosensor based on ZnO nanoparticles. The photovoltaic effect of ZnO nanoparticles can greatly enhance the catalytic activity of AChE and then improve the performance of enzyme electrode. Based on the notable change in voltammetric signal of the AChE biosensor, the simple method for screening of OPs exposure was established. The constructed biosensor processing good fabrication reproducibility, acceptable stability, fast response and low detection limit has potential application in detection of other toxic compounds against to AChE.

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