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Short communication

Development of acetylcholinesterase biosensor based on CdTe quantum dots/gold nanoparticles modified chitosan microspheres interface

Dan Du[a,*], Shizhen Chen[a], Dandan Song[a], Haibing Li[a], Xi Chen[b,*]

[a] Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, China
[b] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China

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ABSTRACT

In this paper, a novel acetylcholinesterase (AChE) biosensor was constructed by modifying glassy carbon electrode with CdTe quantum dots (QDs) and excellent conductive gold nanoparticles (GNPs) though chitosan microspheres to immobilize AChE. Since GNPs have shown widespread use particularly for constructing electrochemical biosensors through their high electron-transfer ability, the combined AChE exhibited high affinity to its substrate and thus a sensitive, fast and cheap method for determination of monocrotophos. The combination of CdTe QDs and GNPs promoted electron transfer and catalyzed the electro-oxidation of thiocholine, thus amplifying the detection sensitivity. This novel biosensing platform based on CdTe QDs–GNPs composite responded even more sensitively than that on CdTe QDs or GNPs alone because of the presence of synergistic effects in CdTe-GNPs film. The inhibition of monocrotophos was proportional to its concentration in two ranges, from 1 to 1000 ng mL^{-1} and from 2 to 15 μg mL^{-1}, with a detection limit of 0.3 ng mL^{-1}. The proposed biosensor showed good precision and reproducibility, acceptable stability and accuracy in garlic samples analysis.

1. Introduction

Recently, the applications based on nanoparticles (NPs) in electrochemistry have attracted wide interest due to their unique optical, electronic and mechanic properties (Wu et al., 2007a,b). Various NPs such as gold (Zotti et al., 2008), silver (Chen et al., 2007) silica (Sun et al., 2006) and quantum dots (QDs) (Zhang et al., 2007) have been extensively used for ultrasensitive optical and electrochemical assays. With unique chemical and physical properties, gold nanoparticles (GNPs) have shown widespread use particularly for constructing electrochemical biosensors through their high electron-transfer ability between biomolecules and electrode surface (Kumar et al., 2007). Willner's group has extensively studied the electrochemical and optical applications of GNPs and the ability to promote the electron transfer (Willner et al., 2005).

Recently many uses of quantum dots (QDs) in biology were reported, such as cellular labeling (Voura et al., 2004; Chen and Gerion, 2004), in vivo tissue imaging (Gao et al., 2004), QDs assay labeling (Goldman et al., 2004; Tan et al., 2007). QDs-based electrochemical bioassay has also become a favorite topic because of inherent miniaturization, high sensitivity, low cost, and low power requirements. Its ability to promote the direct electron transfer between the biomolecules and electrode surfaces was also explored (Huang et al., 2005; Lu et al., 2005).

Chitosan-based materials have been extensively applied as immobilization matrices for preparation of biosensors (Ding et al., 2007; Tkac et al., 2007; Wu et al., 2007a,b). Chitosan could be formulated as films, beads, microspheres and nanoparticles (Giunchedi et al., 1998). More importantly, chitosan micro/nanoparticles could be spontaneously formed through ionic gelation using tripolyphosphate as the precipitating agent, so that the use of organic solvents can be avoided during preparation and loading (Van der Lubben et al., 2001). And the chitosan microsphere/nanoparticles showed improved immobilization capacity for enzyme and good biocompatibility for preserving the activity of immobilized enzyme (Lin et al., 2007).

Acetylcholinesterase (AChE) is the most important target molecule of organophosphates (OP) compounds. In our previous work, an AChE biosensor based on silica sol–gel film assembling GNPs has been proposed. However, the conductivity of silica sol–gel material is not very good (Du et al., 2007a,b). In the present work, we constructed a novel interface with CdTe QDs and excellent conductive GNPs though chitosan microspheres to immobilize AChE, leading to a sensitive method for rapid determination of monocrotophos (Fig. 1). To the best of our knowledge, however, there has been no report focused on the synergy effect between CdTe QDs and GNPs to facilitate electron-transfer processes and the action

* Corresponding authors. Tel.: +86 27 67867953.
E-mail addresses: dudan@mail.ccnu.edu.cn (D. Du), xichen@xmu.edu.cn (X. Chen).

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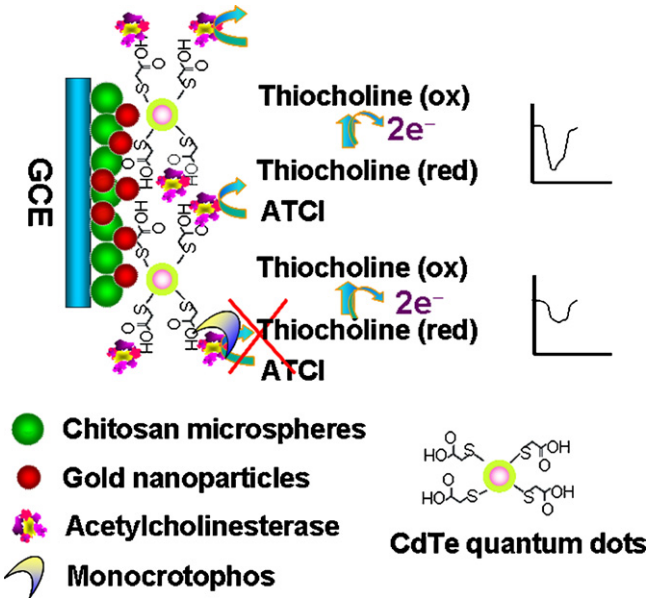

Fig. 1. Schematic diagram for biosensor fabrication and determination of monocrotophos.

of the immobilized AChE for OP determination. This new electrochemical system based on CdTe QDs–GNPs electrode responded even more sensitively than those modified by CdTe QDs or GNPs alone.

2. Experimental

2.1. Reagents

AChE (Type C3389, 500 U mg^{-1} from electric eel), acetylthiocholine chloride (ATCl), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and sodium tripolyphosphate (TPP) were purchased from Sigma–Aldrich (St. Louis, USA) and used as received. UV spectra were recorded using a UV-2501 spectrophotometer (Shimadzu; Kyoto Japan). Monocrotophos and gold (III) chloride hydrate (3.9 g mL^{-1}) were purchased from Treechem Co. (Shanghai, China). 5,5,-Bithiobis-2-nitrobenzoate (DTNB) was obtained from Alfa Aesar. 24-nm-diameter gold nanoparticles (GNPs) were prepared and characterized with transmission electron microscopy as described before (Liu and Ju, 2002). Te powder and thioglycolic acid (TGA) were purchased from Acros Organics (Geel, Belgium). $CdCl_2$, $NaHB_4$, chitosan (95% deacetylation), phosphate buffer solution (PBS) and other reagents were of analytical reagent grade. Aqueous solutions were prepared with double distilled water.

2.2. Preparation of CdTe QDs

CdTe QDs were prepared according to the method described elsewhere (Gao et al., 1998). Finally, the CdTe QDs functionalized with carboxyl groups were dispersed in PBS and its final concentration was 5 mM. CdTe QDs appeared as spherical particles under TEM but tended to partially aggregate (Fig. S1). The aggregation may be induced by excess washing before the sample preparation for TEM measurement. Therefore, only the isolated QDs in the TEM image were used in the size measurement. The average diameter of CdTe QDs was 3 nm.

2.3. Preparation of chitosan microspheres sol–gel

Chitosan microsphere (CM) sol–gel was prepared via an ion-tropic gelation method (Grenha et al., 2007). Because of the poor solubility of chitosan, the mixture was first vortexed and then filtered through 0.22-μm Millipore syringe filters to remove any impurities. In brief, 3% (w/v) chitosan solution was prepared in 1% (v/v) acetic acid. This chitosan solution was added drop-by-drop to a gently stirred 10% (w/v) sodium tripolyphosphate solution through a 16 gauge needle using a programmable syringe pump at a constant pumping rate of 20 mL h^{-1}. The formed CM was allowed to be cured in the TPP solution for additional 24 h. Microspheres were then washed with double distilled water and air dried for 24 h at room temperature.

2.4. Construction of AChE-CdTe-GNPs-CM/GCE

A glassy carbon electrode (GCE) was activated according to references (Du et al., 2005). 100 μL GNPs were added into 1 mL of CM solution, and this mixture was ultrasonicated for several minutes to form a stable composite. 3.0 μL of the resulting composite was coated on a pretreated GCE and left to react at 20 °C for 4 h. Then it was soaked in 50 μL CdTe QDs solution containing 20 mg mL^{-1} EDC and NHS for 10 h to assemble CdTe QDs. After being washed thoroughly with double distilled water, the resulting electrode was incubated in 50 μL AChE solution for 12 h. AChE was bound to activate CdTe QDs through covalent binding of Schiff bases. Then it was washed with PBS to obtain the AChE-CdTe-GNPs-CM/GCE. The activity of the enzyme was determined by acetylthiocholine hydrolysis to thiocholine and the oxidation of 5,5,-bithiobis-2-nitrobenzoate (DTNB) to yield the yellow 5-thio-2-nitrobenzoic acid, which was detected spectrophotometrically (Ellman et al., 1961). After several rinses with large amounts of PBS, no AChE activity could be detected in the rinsing solutions. The prepared biosensor was stored at 4 °C when not in use. For comparison, AChE-GNPs-CM/GCE, AChE-CdTe-CM/GCE and CdTe-GNPs-CM/GCE electrodes were prepared using a similar procedure.

2.5. Apparatus

The electrochemical measurements were carried out on a CHI 660c electrochemical working station (CH Instruments Co.). A three-electrode system was employed with AChE-CdTe-GNPs-CM/GCE as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. The electrochemical impedance experiment was carried out in 5 mM $Fe(CN)_6^{4-/3-}$ with frequencies ranging from 100 kHz to 0.1 Hz with an amplitude of 5 mV. The static water contact angle was measured at 25 °C by means of an OCA 20 contact angle system (Dataphysics, Germany). A JEOL-JEM 2010 TEM was operated at 200 kV.

2.6. Inhibition measurement using AChE biosensor

The inhibitions of monocrotophos were evaluated as follows:

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

where $i_{\text{P,control}}$ and $i_{\text{P,exp}}$ were the peak currents of 1.0 mM ATCl on AChE-CdTe-GNPs-CM/GCE without and with monocrotophos inhibition, respectively.

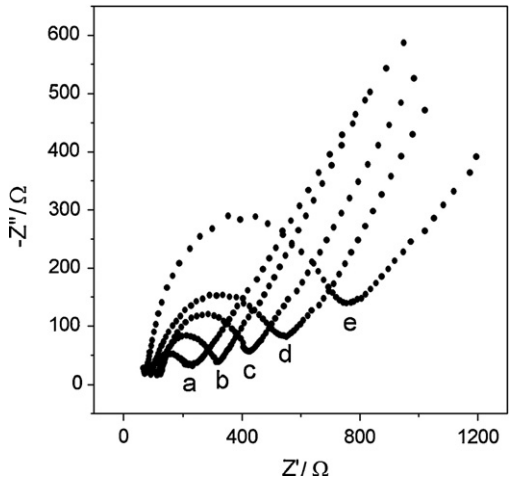

Fig. 2. Electrochemical impedance spectra of (a) CdTe-GNPs-CM/GCE, (b) GNPs-CM/GCE, (c) CdTe-CM/GCE, (d) bare GCE and (e) AChE-CdTe-GNPs-CM/GCE recorded in pH 7.0 PBS containing 50 mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$.

3. Results and discussion

3.1. Surface hydrophilicity

To gain better understanding of modification processes, the surface hydrophilicity was characterized by measuring their contact angles, as shown in Fig.S2 The CM/GCE displayed a small decrease of contact angle and thus a better hydrophilic surface than bare GCE. However, the obtained GNPs-CM/GCE led to an obvious decrease of contact angle, indicating a remarkable improvement of surface hydrophilicity due to unique chemical and physical properties of GNPs. Subsequent assembly of CdTe QDs on the electrode surface resulted in a continuous decrease of contact angle because of large amount of carboxyl groups around CdTe molecules. The resulting hydrophilic surface provided an excellent microenvironment for enzyme adhesion (Du et al., 2007a,b).

3.2. Electrochemical impedance analysis

Electrochemical impedance spectra (EIS) were performed to provide further information on the impedance changes of the electrode surface in the modification process. Curve d in Fig. 2 showed the EIS plot on the bare GCE, which was 600 Ω. After treated with GNPs-CM, the EIS of the GNPs-CM/GCE resulted in an apparent decrease of interfacial resistance due to the excellent electrical conductivity of GNPs. This was reflected by the decrease of the semicircle part on the spectrum (curve b). And then, when CdTe QDs were assembled onto the GNPs-CM/GCE, we were surprised to find that the interfacial resistance of CdTe-GNPs-CM/GCE led to a further decrease (curve a). The reason may be the presence of synergistic effects in CdTe-GNPs film. The combination of GNPs and CdTe QDs played an important role in the electronic transport, which made it easier for electron transfer than that in GNPs or CdTe QDs alone. For comparison, if CdTe was directly assembled onto CM/GCE, the obtained CdTe-CM/GCE displayed higher resistance (curve c) than CdTe-GNPs-CM/GCE (curve a) and GNPs-CM/GCE (curve b), and lower resistance than bare GCE (curve d). Although CdTe QDs have the ability to promote electron transfer, it is one kind of semiconductors and its conductivity is not good enough (Liu et al., 2007). Therefore, CdTe-GNPs-CM composite film was used for following experiment. However, after AChE was immobilized on the electrode, the interfacial resistance increased remarkably (curve e) due to the increase of the thickness of the interface.

3.3. UV–vis spectrum

The position of Soret absorption band can provide detailed information about conformation of protein (Shi et al., 2007). Solution-phase AChE displayed a narrow Soret band at 406 nm. After immobilization of AChE on the surface of the AChE-CdTe-GNPs-CM/GCE, an obvious Soret band without shift was clearly seen, indicating that AChE has been successfully immobilized on the electrode surface and no significant denaturalization occurred.

On the basis of the results of surface hydrophilicity, EIS, UV–vis spectrum and the cyclic voltammetric behavior (the electrochemical activity peaks of the sensors scaled linearly with the scan rate), we concluded that GNPs, CdTe QDs and AChE have been successfully immobilized on the electrode surface and thus the biosensor has been fabricated.

3.4. Cyclic voltammetric behavior of the biosensor

Fig. 3A showed the cyclic voltammograms of ATCl at different electrodes. No peak was observed at GCE (curve a) and AChE-CdTe-GNPs-CM/GCE (curve b) in pH 7.0 PBS. When 1.0 mM ATCl was added into PBS, the cyclic voltammogram of AChE-CdTe-GNPs-CM/GCE showed an irreversible oxidation peak at 585 mV (curve

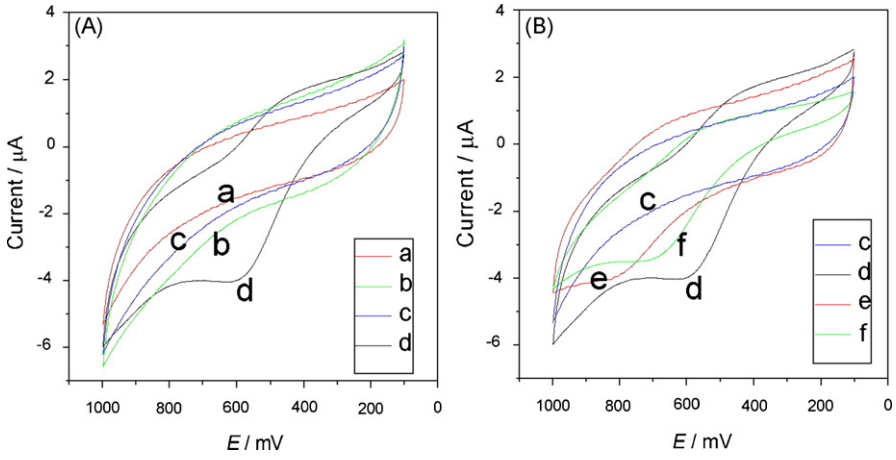

Fig. 3. (A) Cyclic voltammograms of (a) bare GCE and (b) AChE-CdTe-GNPs-CM/GCE in pH 7.0 PBS; and (c) CdTe-GNPs-CM/GCE, (d) AChE-CdTe-GNPs-CM/GCE in pH 7.0 PBS containing 1.0 mM ATCl. (B) Cyclic voltammograms of (d) AChE-CdTe-GNPs-CM/GCE, (e) AChE-CdTe-CM/GCE and (f) AChE-GNPs-CM/GCE in pH 7.0 PBS containing 1.0 mM ATCl.

d), while no detectable signal was observed at CdTe-GNPs-CM/GCE without immobilization of AChE (curve c). Obviously this peak came from the oxidation of thiocholine, hydrolysis product of ATCl, catalyzed by immobilized AChE.

For comparison, the cyclic voltammetric behaviors of ATCl at AChE-CdTe-CM/GCE and AChE-GNPs-CM/GCE were also studied. As shown in Fig. 3B, although an obvious oxidation peak at 826 mV on AChE-CdTe-CM/GCE (curve e) and 689 mV on AChE-GNPs-CM/GCE (curve f), respectively, could be seen, the peak currents were much lower and the peak potentials shifted positively compared to those on the AChE-CdTe-GNPs-CM/GCE (curve d). This phenomenon indicates that CdTe QDs have the ability to promote the electron transfer of enzyme, but its inherent conductive properties and catalytic behavior is not as good as GNPs. GNPs played an important role in facilitating the electron transfer between the enzyme and the electrode surface. However, the combination of GNPs and CdTe QDs further improved the electronic transport may be due to the presence of synergistic effects in CdTe-GNPs film. This composite film made it easier for electron transfer than that in GNPs or CdTe QDs alone.

The peak current increased and the peak potential shifted slightly with increasing scan rate. The peak currents exhibited a linear dependence on the scan rate ranging from 10 to 200 mV s^{-1}, indicating a typical surface-controlled electrode process. According to Laviron's treatment, the heterogeneous rate constant of electron transfer was 1.517 s^{-1}. This value showed a relatively fast electron transfer. For the freely diffusing enzyme, the peak currents exhibited a linear dependence on the square root of scan rate, indicating a typical diffusion-controlled process. Since AChE was normally immobilized on the electrode by amine coupling using EDC and NHS, it is not easily left free in solution due to the tightly covalent bound.

3.5. Inhibition measurements and recovery analysis

Monocrotophos, a kind of organophosphorous pesticides, is known to be involved in the irreversible inhibition on AChE, thus reduced the enzymatic activity to its substrate.

The optimum incubation time of 8 min was observed (Fig. S3). With the conditions as specified above, the inhibition of monocrotophos was proportional to its concentration in two ranges, from 1 to 1000 ng mL^{-1} and from 2 to 15 μg mL^{-1} (Fig. S4), with the correlation coefficients 0.9927 of and 0.9945, respectively. The calibration sensitivity was 36.07 and 0.035%/μg mL^{-1}, respectively. The IC50 and IC10 were 1.97 and 0.093 μg mL^{-1}. The detection limit was 0.3 ng mL^{-1}, comparable to 0.9 ng mL^{-1} at 3-mercaptopropionic acid SAM electrode (Pedrosa et al., 2007), lower than those of 5 ng mL^{-1} at electrodeposited chitosan layer (Du et al., 2007a,b).

To further demonstrate the practicality of the proposed biosensor, the recovery test was studied by adding different amounts of monocrotophos into garlic samples of 0.1 M PBS. The recoveries were from 95.0% to 102.3%. The average precision was ±6.0% (Table 1 in supporting information). The results indicated that the proposed method was highly accurate, precise and reproducible. It can be used for direct analysis of practical samples.

3.6. Precision of measurements and stability of biosensor

The intra-assay precision of the biosensors was evaluated by assaying one enzyme electrode for eight replicate determinations in 1.0 mM ATCl after being treated with 500 ng mL^{-1} monocrotophos solutions for 8 min. Similarly, the inter-assay precision, or fabrication reproducibility, was estimated at eight different electrodes. The RSD of intra-assay and inter-assay were found to be 4.1% and 5.3%, respectively, indicating acceptable precision and reproducibility. When the enzyme electrode was not in use, it was stored at 4 °C in dry condition. No obvious decrease in the response of ATCl was observed in the first 10-day storage. After a 30-day storage period, the sensor retained 92% of its initial current response.

4. Conclusions

A simple enzyme biosensor based on immobilization of AChE on CdTe QDs–GNPs composite modified chitosan microspheres interface was developed for the amperometric determination of organophosphate pesticide. The formation of CdTe QDs–GNPs composite not only favored the interface enzymatic hydrolysis reaction to form electroactive substance, increasing the sensitivity and facilitating the amperometric response of the biosensor but also prevented enzyme molecule from leaking out of the electrode through covalent binding of Schiff bases, which was based on the interactions between amine groups of AChE and carboxyl groups of QDs. The performance of the resulting electrode was improved greatly compared with that of electrode modified by CdTe QDs or GNPs alone. We are currently facing the disadvantage of the low selectivity of AChE because the degree of inhibition on AChE by organophosphate pesticide is almost the same. However, this does not necessarily compromise its value based on this mode. The ease of performance and the cost effectiveness of the system show the high potentiality in total amount of pesticide analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.05.005.

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