Journal of Electroanalytical Chemistry 740 (2015) 8–13

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Effective amperometric biosensor for carbaryl detection based on covalent immobilization acetylcholinesterase on multiwall carbon nanotubes/graphene oxide nanoribbons nanostructure

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ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form 22 December 2014
Accepted 24 December 2014
Available online 2 January 2015

Keywords:
Acetylcholinesterase
Covalent immobilization
Graphene nanoribbon
Pesticide
Biosensor

ABSTRACT

This work described a sensitive electrochemical biosensor for the detection of carbaryl pesticide based on covalent immobilization of acetylcholinesterase (AChE) on the multiwall carbon nanotubes/graphene oxide nanoribbons (MWCNTs/GONRs) nanostructure. The catalytic activity of AChE immobilized on the MWCNTs/GONRs film was superior to that of MWCNTs due to the covalent binding technique, and the biosensor showed high affinity to acetylthiocholine with a Michaelis–Menten constant value of 0.25 mM. Based on the inhibition of carbaryl on the enzymatic activity of the immobilized AChE, the resulting biosensor exhibited superior performance for carbaryl detection including good reproducibility, acceptable stability, and wide linear range from 5 nM up to 5000 nM with a low detection limit of 1.7 nM. The biosensor was successfully challenged with real sample demonstrating to be a useful analytical tool for insecticide detection.

1. Introduction

Pesticides determination has attracted great attention during recent years because pesticides exhibit high acute toxicity and can cause long-term damage to both the environment and lives even at trace levels [1]. Therefore, it has become a key activity for continuous monitoring low pesticide levels in food, water, and air. Although traditional chromatographic methods (including high-performance liquid chromatography, capillary electrophoresis, and mass spectrometry) are effective for the analysis of pesticides, they have certain limitations such as complexity, time-consuming sample preparation, and the requirement of expensive apparatus and trained persons to operate [2,3]. Over the past years, acetylcholinesterase (AChE) inhibition-based biosensors have emerged as simple, rapid, and ultra-sensitive tools for pesticide analysis [4,5]. The development of an AChE biosensor requires the immobilization of AChE on the transducer and thus, the immobilization is a key point to obtain a sensitive biosensor. Several techniques for immobilizing enzymes on transducers have been reported in previous literature, such as physical adsorption [6], entrapment [7] and cross-linking [8]. Unfortunately, these immobilization methods allow enzyme leakage, thus resulting in reduced stability of the enzyme electrode [4]. In order to avoid these drawbacks, covalent enzyme immobilization has been reported as a successfully alternative to fabricate biosensors for better biomolecule activity and greater stability [9–11].

As a novel graphene-based functionalized nanomaterial, graphene/carbon nanotube hybrid structure has sparked much research interests in the past two years because of its excellent properties such as flexibility, stretchability and electrical conductivity [12,13], which could generate new potential in the fields from material science to electrochemical sensor [14–17]. For instance, by virtue of the synergistic effect of three-dimensional multi-walled carbon nanotubes (MWNTs)–graphene, Pradhan's group discovered that the mechanical and thermal properties of high-performance silicone rubber were enhanced significantly [14]. CTAB-functionalized graphene oxide (CTAB–GO) and MWNTs have been used to construct sensing interface to achieve simultaneous detection of dopamine, ascorbic acid, uric acid, and NO_2^- by Yang's group, and the results indicated that due to the synergistic integration of the CTAB–GO and MWNTs, the CTAB–GO/MWNTs/GCE could facilitate the simultaneous determinations of the analytes with high sensitivity and selectivity compared with MWNTs/GCE and CTAB–GO/GCE [15]. According to Lu's report, Pt–Pd NPs/MWNTs–rGO has been synthesized for the fabrication of electrochemical sensor, and the results indicated that the Pt–Pd NPs/MWNTs–rGO catalyst exhibited higher electrocatalytic activities and high sensitivity than the Pt–Pd NPs/rGO and Pt–Pd NPs/

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http://dx.doi.org/10.1016/j.jelechem.2014.12.037
1572-6657/

MWNTs due to the excellent electric conductivity and large surface-to-volume ratio of the Pt–Pd NPs/CNTs–rGO film [16]. All these results above indicate that the graphene/carbon nanotube hybrid structure shows grand potential in various fields especially for fabricating electrochemical sensing interface, and up to now, it is just the beginning of this fantastic topic.

Most recently, Sun et al. synthesized the MWCNTs/graphene oxide nanoribbons (MWCNTs/GONRs) by a unique microwave-assisted unzipping of MWCNTs, which provided superior oxidation currents for the analytes compared with those of MWCNTs and graphene [18]. Inspired by these insights, herein a covalent binding strategy for immobilization of AChE on a MWCNTs/GONRs modified electrode was described and an amperometric biosensor for determination of pesticides carbaryl in water samples was constructed. The prepared electrochemical sensor was characterized by electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammetry (DPV). As expected, the electrochemical sensor not only can strikingly improve the sensitivity for carbaryl analysis, but also obtains good stability and thus can be potentially exploited for the detection of pesticide residuals or other deleterious chemicals in environment.

2. Experimental

2.1. Reagents

MWCNTs ($\Phi \sim 10$ nm) were obtained from Shenzhen Nanotech Port Co. Ltd. Acetylcholinesterase (AChE, Type C3389, 500 U mg^{-1} from electric eel), acetylthiocholine chloride (ATCl) and carbaryl were purchased from Sigma–Aldrich (USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) were purchased from J&K Scientific Ltd. 0.1 M phosphate buffer solution (PBS, pH 7.0) was prepared by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4, and adjusting the pH with 0.1 M NaOH. Other chemicals were of analytical grade and used without further purification, and all solutions were prepared with doubly distilled water.

2.2. Apparatus

Transmission electron microscopy (TEM) image was taken with a JEOL 2100 TEM (JEOL, Japan) operated at 200 kV, and the FT-IR spectra of the samples were obtained from FT-IR Spectrometer (Nicolet Nexus 470 FT-IR). All the electrochemical experiments were performed with a CHI660 B electrochemical analyzer (Chen Hua Instruments, Shanghai, China) with a conventional three-electrode system where glassy carbon electrode (GCE, $\Phi = 3$ mm) was used as working electrode, a saturated calomel electrode (SCE) as reference electrode and platinum wire as counter electrode, respectively. EIS was performed in a 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-/4-}$ with a frequency range from 0.1 Hz to 10 kHz at 0.2 V, and the amplitude of the applied sine wave potential in each case was 5 mV which was taken with a ZENNIUM electrochemical workstation (Zahner Instruments, Germany).

2.3. Preparation of MWCNTs/GONRs

MWCNTs/GONRs was prepared according to previous report with modifications [19]: 120 mg of MWCNTs was suspended in 40 mL of H_2SO_4/H_3PO_4 (9:1), and the mixture was allowed to stir 1 h before the addition of $KMnO_4$ (600 mg). The reaction mixture was heated at 65 °C for 2 h, and then was poured onto 400 mL of ice containing H_2O_2 (30%, 5 mL). The solution was filtered over a polytetrafluoroethylene membrane, and the remaining solid was washed with acidic water followed by ethanol.

2.4. Preparation of the modified electrodes

Prior to modification, the GCE was first polished with sand paper followed by 1.0, 0.3, and 0.05 μm alumina slurry, respectively. After successive sonication in ethanol and double distilled water, the electrode was rinsed with double distilled water and allowed to dry at room temperature. As displayed in Scheme 1, the modified electrode was prepared as follows: initially, the pretreated GCE was modified by dropping 6 μL of 0.5 mg mL^{-1} MWCNTs/GONRs/water solution and drying (denoted as MWCNTs/GONRs/GCE), then 0.1 M PBS (pH 7.4, 10 μL) containing NHS (0.005 M) and EDC (0.01 M) was casted on the MWCNTs/GONRs/GCE and incubated at 25 °C for 6 h. After rinsing once with PBS and twice with distilled water, the electrode was coated with 4 μL AChE solutions (500 U mL^{-1}), which were incubated at 25 °C for 30 min. After evaporation of water, the modified electrode was washed with 0.1 M PBS to remove the unbound AChE, and the resulting AChE–MWCNTs/GONRs/GCE was stored at 4 °C when not use. In addition, AChE–MWCNTs/GCE was prepared for comparison.

2.5. Measurement procedure

The principle of the amperometric electrochemical biosensor based on AChE inhibition was shown in Scheme 1. When AChE was immobilized on the working electrode surface, its interactions with the substrate ATCl produced the electroactive product of thiocholine. In the absence of the pesticide (inhibitor), the thiocholine could be oxidized at an appropriate applied voltage; while in the presence of the pesticide, the conversion of ATCl was decreased, and thus the anodic oxidation current of thiocholine was decreased.

Inhibition of pesticide: the proposed AChE–MWCNTs/GONRs/GCE was first immersed in 0.1 M PBS containing different concentrations of standard carbaryl solution for 3 min and then transferred to the electrochemical cell of 5.0 mL PBS containing 1.0 mM ATCl to study the electrochemical response by DPV between 0.2 and 1.0 V (vs. SCE). The inhibition of pesticide was calculated as follows: Inhibition (%) = $(1 - I_{p,exp}/I_{p,control}) \times 100$, where $I_{p,control}$ is the peak current of ATCl on AChE–MWCNTs/GONRs/GCE and $I_{p,exp}$ is the corresponding peak current of ATCl with pesticide inhibition.

2.6. Preparation and determination of real samples

The application of AChE–MWCNTs/GONRs/GCE has been evaluated by real sample analysis using cabbage samples. Two samples of 2 g each was kept in distilled water spiked with 100 and 250 nM of carbaryl, respectively. After a standing time of 48 h, the two samples were extracted with 15 mL of ether. The supernatants were then filtered through a 0.45 μm membrane and then evaporated to dryness. About 2 mL of ethanol was added to the dry residue and diluted to 100 mL with 0.1 M PBS (pH 7.0) for determination.

3. Results and discussion

3.1. Characterization of MWCNTs/GONRs

The morphology of pristine MWCNTs and the resulting MWCNTs/GONRs was characterized by TEM, as shown in Fig. 1. The clear edge of MWCNTs with the average diameter of ~10 nm could be observed in Fig. 1A. For comparison, Fig. 1B illustrates that the rough edge of graphene structures were found on both sides of the nanotubes, whereas the central cores of nanotubes remained tubelike, and this type of core–shell structure was termed as a