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Amperometric choline biosensors based on multi-wall carbon nanotubes and layer-by-layer assembly of multilayer films composed of Poly(diallyldimethylammonium chloride) and choline oxidase

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ABSTRACT

A layer-by-layer deposition technique combined with Multi-wall carbon nanotubes (MWCNTs) was employed for fabricating choline sensors. The terminals and side-walls were linked with oxygen-containing groups when MWCNTs were treated with concentrated acid mixtures. A film of MWCNTs was initially prepared on the platinum electrode surface. Based on the electrostatic interaction between positively charged polyallylamine (PAA) and negatively charged MWCNTs and poly(vinyl sulfate) (PVS), a polymer film of $(PVS/PAA)_3$ was alternately adsorbed on the modified electrode continuously to be used as a permselective layer. Then poly(diallyldimethylammonium) (PDDA) and choline oxidase(ChOx) multilayer films were assembled layer-by-layer on the pretreated electrode, so an amplified biosensor toward choline was constructed. The choline sensor showed a linear response range of 5×10^{-7} to 1×10^{-4} M with a detection limit of 2×10^{-7} M estimated at a signal-to-noise ratio of 3, and a sensitivity of 12.53 μA/mM with a response time of 7.6 s in the presence of MWCNTs. Moreover, it exhibited excellent reproducibility, long-term stability as well as good suppression of interference. This protocol could be used to immobilize other enzymes for biosensor fabrication.

1. Introduction

Carbon nanotubes (CNTs) represent a new kind of the carbon derivatives and have been widely recognized as the quintessential nanomaterial since their discovery in 1991 [1]. It is found that CNTs possess many unique properties such as good electrical conductivity, strong adsorptive ability, excellent bioconsistency and high strength [2], and also provide a high electrocatalytic effect and a fast electron transfer rate [3–5], which make CNTs very promising in the design of electrochemical sensors and biosensors [6–8]. Although the poor solubility of CNTs in most solvents is a key limitation to construct CNTs-based electronic devices, considerable efforts have been made for their successful application in biosensors.

The oxidation procedure used for purification of CNTs, mainly to remove metal catalysts and amorphous carbon, also results in partial oxidation of the carbon atoms to produce oxygen-containing groups (e.g., carboxylic acid groups), especially in the open ends of the nanotubes [9,10]. CNTs with the produced groups are negatively charged in aqueous solution and can interact with positively charged polyelectrolytes [11] or biomolecules. In addition, the acid treatment shortens the chain lengths of CNTs, thereby enabling them to be immobilized easily and stably on an electrode [12].

Choline is a precursor for the biosynthesis of an important neurotransmitter—acetylcholine in both of the peripheral and central nervous systems of mammals [13]. It is often used as a marker of cholinergic activity in the brain tissue, especially in the field of clinic detection of a disease, such as Parkinson's and Alzheimer's diseases [14–16]. Therefore, its determination is important in biological and clinical detection, and the development of amperometric choline biosensors utilizing choline oxidase (ChOx) is an active research area.

Enzyme immobilization technology has attracted much attention in connection with the design, fabrication and applications of biosensors. The catalytic characteristics as well as the stability and sensitivity of immobilized enzymes are influenced by immobilization methods and support materials. ChOx has been immobilized onto the electrode surfaces by a variety of techniques, including casting, aqueous sol–gel and electropolymerization, cross-linking and self-assembly techniques [17–27]. Recently, ordered protein multilayers can also be produced by using the layer-by-layer (LBL) deposition technique, which involves an alternate adsorption of anionic and cationic polyelectrolytes from solution onto a solid surface through electrostatic force of attraction [28–34]. Thin films containing enzymes can be also prepared by this technique and the film-modified electrodes have been shown to exhibit good reproducibility, stability and high sensitivity [29–34].

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Enzyme-based biosensors for indirect detection of the enzyme substrates such as glucose, cholesterol, and choline often involve monitoring the formation of H_2O_2 in enzymatic reactions. The high applied potential required for H_2O_2 oxidation is problematic as the resulting sensor is susceptible to interferences, and then very efficient permselective polymer layers are required to reject electroactive interferences such as ascorbic acid, uric acid while maintaining a high permeability to smaller molecules such as H_2O_2. In this respect, three layers of poly(vinyl sulfate)/polyallylamine (PVS/PAA) (hereafter denoted as $(PVS/PAA)_3$)were assembled onto the electrode surface before enzyme immobilization. The polymer film of $(PVS/PAA)_3$ is reported to be effective at eliminating the influence of electroactive compounds on the sensor response [29].

In this paper, a choline oxidase electrode was prepared to construct the sensor with a three-step modification process. In the first step, a platinum (Pt) electrode was modified with multi-wall carbon nanotubes (MWCNTs). Second, the modified electrode was coated with a thin film of $(PAA/PVS)_3$. Third, (ChOx/Poly(diallyldimethylammonium))n (denoted as (ChOx/PDDA)n) film was immobilized on the pretreated electrode. The advantage of this choline sensor was to use a simple procedure to modify the electrode surface by MWCNTs which act as the physicochemical transducer, employing $(PVS/PAA)_3$ film to promote the anti-interference ability of the electrode, throughout the stable immobilization of enzymes using the LBL technique. To the best of our knowledge, it was a new try in choline biosensor construction which combined well the advantages of MWCNTs and LBL technique. In the following sections, the electrochemical characteristics and interference effects of the choline sensor were investigated, in comparison with the sensor made without MWCNTs and the sensor without $(PVS/PAA)_3$ film, respectively. Stability and selectivity were also studied.

2. Experimental

2.1. Reagents

Poly(diallyldimethylammonium chloride)(PDDA, *MW*: 40,000–50,000), poly (allylamine)(PAA, *MW*:10,000) and poly(vinyl sulfate, potassium salt) (PVS, *MW*:170,000) were purchased from Acros. Co. The chemical structures of the polymeric materials are shown in Fig. 1. Choline oxidase (ChOx, EC 1.1.1.17, from Alcaligenes species, 14 units/mg) was purchased from Sigma Chemical Co. Multi-wall carbon nanotubes (>50 nm diameter and 0.5–1 μm length), with 95% purity, were obtained from Institute of Organic Chemistry, Chinese Academy of Sciences (ChengDu, China). Hydrogen peroxide (30%,v/v aqueous solution), and choline chloride were obtained from Beijing Chemical Reagent Company. All other reagents were of analytical grade and were used without further purification.

2.2. Apparatus and electrochemical measurements

The electrochemical instrument consisted of a 283 Potentiostat–Galvanostat (EG&G PARC with a software M270). The electrochemical response of the choline sensor was measured with a conventional three-electrode electroanalysis system. The PDDA/ChOx film-modified electrode (3 mm diameter) was used as a working electrode with an Ag/AgCl electrode (saturated KCl) as a reference electrode and a platinum spiral wire (1 mm diameter) as an auxiliary electrode. The amperometric measurement of the electrode was carried out at +0.6 V versus Ag/AgCl in a solution stirred with a magnetic stirrer at 600 rpm. All the chronoamperometric measurements were carried out in a 0.1 M phosphate buffer solution (PBS, pH 8.0). Aliquots of the choline solution were successively pipetted to the supporting solution and the current response was recorded stepwise after the electrode background current became stable.

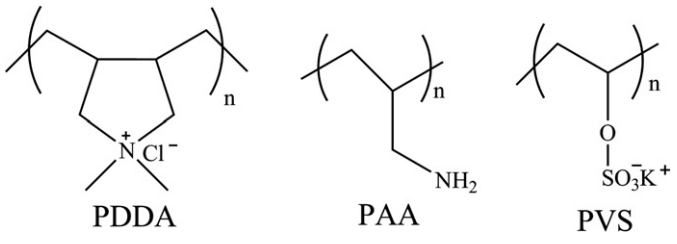

Fig. 1. Molecule structure of PDDA, PAA and PVS.

2.3. Construction of the choline biosensor

2.3.1. Preparation of electrode modified with MWCNTs

The platinum electrode was hand-polished with emery paper and alumina slurry(0.05 μm diameter), and then ultrasonically cleaned in double-distilled water. 1 mg of MWCNTs were dispersed in a 3:1 (v/v) mixture of concentrated sulfuric and nitric acid with the aid of ultrasonic agitation for about 4 h, forming a 1 mg/ml black solution, and shortened MWCNTs with terminal COOH groups were produced [35,36]. Then the carbon nanotubes were separated by centrifugation (10,000 rpm), and washed with distilled water by centrifugation until the pH of the resulting solution became neutral. The MWCNTs film was prepared by immersing the Pt electrode in a solution of MWCNTs for 25 min to immobilize MWCNTs on the surface, and the adsorption was responsible of MWCNTs immobilization. After modification, the electrode was rinsed in PBS for 5 min to remove the loosely adsorbed carbon nanotubes.

2.3.2. Immobilization of choline oxidase at LBL composite films on the surface of the modified electrode

ChOx can be used as polyanionic material for preparing LBL film around pH 8.0, because the isoelectric point (pI) of ChOx lies around pH 4.5. The pH of ChOx solution has been set apart from isoelectric point so that the protein is sufficiently charged. Thus, the enzyme solution for immobilization was prepared with 2 mg/ml of ChOx in 0.1 M PBS at pH 8.0. The thin films were prepared on the surface of the Pt electrode according to the reported procedure [29]. Polycation PAA has the amino groups, as a highly positively charged specie, which can be chemisorbed onto the −COOH groups of MWCNTs with the electrostatic adsorption between them, so the MWCNTs/Pt electrode was firstly immersed in PAA solution for 25 min and rinsed in PBS for 5 min, then the PAA/MWCNTs/Pt electrode was transferred to polyanion PVS solution for 25 min and rinsed in PBS for 5 min. The above adsorption and rinsing processes were repeated three times to prepare $(PVS/PAA)_3$ film on the MWCNTs/Pt electrode. Then, the pretreated electrode was immersed in PDDA (2 mg/ml) for 25 min to deposit the polycationic layer. After being rinsed with PBS to remove any weakly adsorbed polycation, the electrode was immersed in the ChOx solution for 25 min to immobilize the enzyme on the polycation-modified electrode through electrostatic force. Such alternate deposition was carried out repeatedly to obtain the desired number of layers [37]. Thus, the resulting electrode (hereafter denoted as (ChOx/PDDA)n/$(PVS/PAA)_3$/MWCNTs/Pt) together with the enzyme electrode without MWCNTs (hereafter denoted as (ChOx/PDDA)n/$(PVS/PAA)_3$/Pt) was used for the electrochemical measurements later.

3. Results and discussion

3.1. Electrochemical properties of modified electrode

Fig. 2 compares amperometric responses to 1.0×10^{-3} M hydrogen peroxide (H_2O_2)at (a) MWCNTs-modified and (b) bare electrode under the conditions that the potential was kept at 0.6 V in a 0.1 M phosphate buffer solution (pH 8.0). As can be seen in the figure, with the addition of H_2O_2, the current increased obviously for both electrodes (a and b).