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A novel tyrosinase biosensor based on chitosan-carbon-coated nickel nanocomposite film

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ABSTRACT

A novel nanocomposite film of tyrosinase–chitosan–carbon-coated nickel nanoparticles (CNi) had been constructed for the detection of catechol. The tyrosinase–chitosan–CNi bionanocomposite film was characterized with scanning electron microscopic (SEM) and electrochemical impedance spectroscopy (EIS). In pH 6.5 phosphate buffer solutions (PBS), the biosensor was applied to detect catechol with a broad linear range from 0.25 nM to 27 μM, the detection limit was brought down to 0.083 nM (S/N = 3). The proposed biosensor demonstrated rapid response, as well as good reproducibility and stability. The chitosan–CNi film was propitious to the immobilization of tyrosinase and to the retention of its bioactivity to a large extent. Therefore, the film has potential applications in the immobilization of other enzyme-based biosensors.

1. Introduction

Phenolic compounds are broadly used in wood preservatives, textiles, herbicides and pesticides, and thus readily release into the ground and surface water [1]. Such compounds are polluting chemicals which can be easily absorbed by animals and human body through skin and mucous membrane [2]. Some of these phenolic compounds have been related with health problems and environmental pollution because of their inherent toxicity [3]. Hence, it is of great importance in environment analysis to closely monitor the quantity of these phenolic compounds. Here we reported the establishment of a simple, fast and sensitive method for phenolic compound detection and measurement.

In recent years, the amount of novel cell biological results have been obtained by the method of electrochemical operations. There are mass reports regarding the direct (mediatorless) electron transfer (ET) reaction of glucose oxidase [4], hemoglobin [5,6] and ferritin [7] at the modified electrodes. Various tyrosinase biosensors have been developed for the detection of phenolic compounds [1,8,9]. López et al. have constructed an enzymatic amperometric biosensor based on biocompatible calcium phosphate cement [8]. Zhang et al. have exploited to immobilize tyrosinase into polyaniline–ionic liquid–carbon nanofiber composite [1]. Zejli and co-workers have prepared the biosensor based on Sonogel-Carbon transducer with tyrosinase alumina sol–gel immobilization [9]. More recently, Labus et el [10] have carried out a systematic study of the covalent immobilization of tyrosinase on mantailored cellulose-based carriers with different pore structure and functionalization. Increased activity and thermal stability of the immobilized enzyme could be achieved. They also found that tyrosinase immobilized on the selected carrier showed increased susceptibility towards suicide inactivation in the presence of diphenols such as catechol. Effective immobilization of enzyme onto the electrode surface is one of the key steps in the fabrications of biosensors. But the fabrication of the referred modified electrodes remained comparably complicated. Therefore, the field is active searching for biosensor with simple fabrication process and excellent performance.

Recently, magnetic nanoparticles as new materials have drawn a lot attention in various fields. Due to their special properties and protective coatings against environmental degradation, the application of the magnetic nanocapsules ranges from immobilization of proteins [11], detection of DNA hybridization [12], drug delivery [13] to immunology [14]. We focused on a new class of magnetic nano-materials, carbon-coated metal crystals, which was first synthesized in 1993 [15] as a nanocrystallite of transitional metal or metal carbide wrapped in graphitic-like carbon [16]. Carbon-coated nickel nanoparticle (CNi) has been chosen as it has special features due to more specific of Ni when compared with carbon onions consisting only of carbon layers [17]. Moreover, with the protection of the outer multilayered graphite, the entrapped metals would not be hydrolyzed and oxidized even after exposure in air for several years, so it is considerably stable and can overcome the agglomeration of nanoparticles. In our previous work, it was also found that CNi modified electrode could accelerate the ET rate and show excellent electrocatalytic activity for some micromoleculars [18]. Here CNi has been applied for the immobilization of macromolecule.

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In this work, a new tyrosinase–chitosan–CNi nanocomposite film was prepared for monitoring catechol. The electrochemical properties of this biosensor were investigated. The new biosensor exhibited rapid response (within 8 s), low detection limit, and broad linear range. The proposed strategy can be extended for the development of other enzyme-based biosensors.

2. Experimental

2.1. Chemicals and reagents

Tyrosinase (EC 1.14.18.1, 5370 U mg^{-1} from mushroom) and chitosan were purchased from Sigma-Aldrich (St. Louis, Missouri). CNi (the average diameter 10–50 nm and nickel metal content 64.5%) was obtained from Professor Xungao Zhang (the Center for Analysis and Measurement, Wuhan University, Wuhan, China). It was synthesized by an alternative current (AC) arc discharge method [16]. Catechol was purchased from Goodtime Bio-Technology Co., Ltd (Wuhan, China). Phosphate buffer was comprised with Na_2HPO_4 and NaH_2PO_4. The pH values were adjusted with NaOH and H_3PO_4. All other reagents were of analytical grade and were used as received without further purification.

2.2. Apparatus

All electrochemical measurements were carried out by a model CHI 660A electrochemical workstation (Chenhua Instrument Company of Shanghai, China), which was PC controlled. A three-electrode system was used in the measurements, with film modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode. All potentials were reported vs. SCE.

Ultrasonic cleaner (Branson 2000, USA) was used for cleaning the electrodes. The pH values of solutions were determined by 320-S acidometer (Mettler-Toledo, Switzerland). The morphologies of the nanocomposite film were observed by scanning electron microscopy (SEM, Philips XL30 TMP). All the data were obtained at room temperature.

2.3. Fabrication of tyrosinase–chitosan–CNi biosensor

Tyrosinase solution with a concentration of 5.0 mg mL^{-1} was prepared in 0.10 M PBS (pH 6.5). Chitosan solution with a concentration of 2.5 mg mL^{-1} was prepared by dissolving 5.0 mg chitosan in 2.0 mL of 0.10 M acetic acid. The CNi suspension (1 mg mL^{-1}) was prepared by dispersing CNi in doubly distilled water with ultrasonication for about 2 h to achieve a well-dispersed suspension. In order to magnify the amperometric responses of the biosensor, the composition of the tyrosinase–chitosan–CNi were optimized. Typically, CNi, chitosan, and tyrosinase with a volume ratio of 1:1:2 were mixed thoroughly, and 10 μL of this mixture was dropped onto the surface of the cleaned GCE. Then the electrode was dried at room temperature. When not in use, the biosensor was preserved at 4 °C in a dry state.

2.4. Electrochemical measurements

Cyclic voltammetric measurements were performed in PBS at a scan rate of 100 mV s^{-1}. Amperometric measurements were carried out in a magnetically stirred PBS. The desired working potential was applied, and transient currents were allowed to decay to a steady-state value. Electrochemical impedance spectroscopy (EIS) was performed in 0.5 mM $Fe(CN)_6^{3-/4}$-containing 0.5 M KNO_3 solution. The frequency scan range was from 0.05 Hz to 100 kHz and the amplitude was 5 mV.

3. Results and discussion

3.1. Characterization of the tyrosinase–chitosan–CNi film

The microstructure of CNi and the morphology of films were characterized by TEM (data has been shown in Ref. [18]) and SEM, respectively. As can be seen, the globe-like CNi were distributed evenly in the presence of chitosan (Fig. 1A), which suggested that the chitosan would help the CNi disperse in the solution. The enzyme distributed regularly throughout CNi–chitosan after entrapped in this film (Fig. 1B). The tyrosinase–chitosan–CNi film was compact and uniform, which indicated that tyrosinase and CNi have been successfully incorporated in the chitosan film [19].

EIS, which could provide useful information on the impedance changes of the electrode surface during the fabrication process [20], was also used to characterize the properties of the modified electrodes. Fig. 2 presents the EIS of the bare GCE (a); CNi–chitosan/GCE (b); chitosan/GCE (c); tyrosinase–chitosan–CNi/GCE (d) and tyrosinase–chitosan/GCE (e) in 0.5 mM $Fe(CN)_6^{3-/4}$-containing 0.5 M KNO_3 solution. It exhibited an almost straight line with the bare GCE (a). After the GCE was coated by CNi–chitosan film or chitosan film, a small semicircle at high frequencies was obtained, indicating that the electron transfer of the redox probe was obstructed. It was noticed that the interfacial

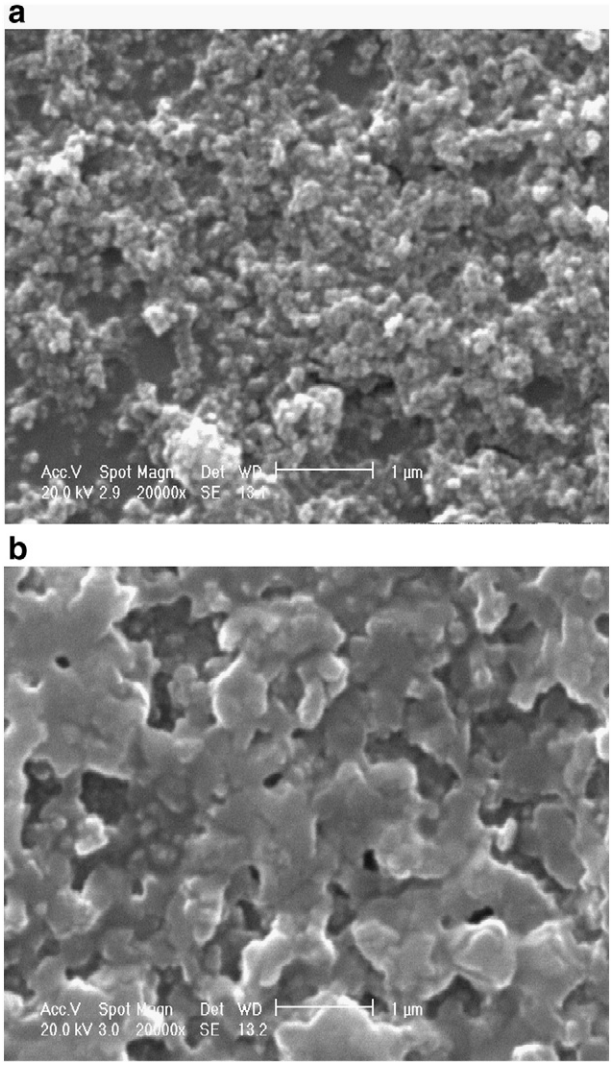

Fig. 1. SEM images of (A) chitosan–CNi film, (B) tyrosinase–chitosan–CNi film.

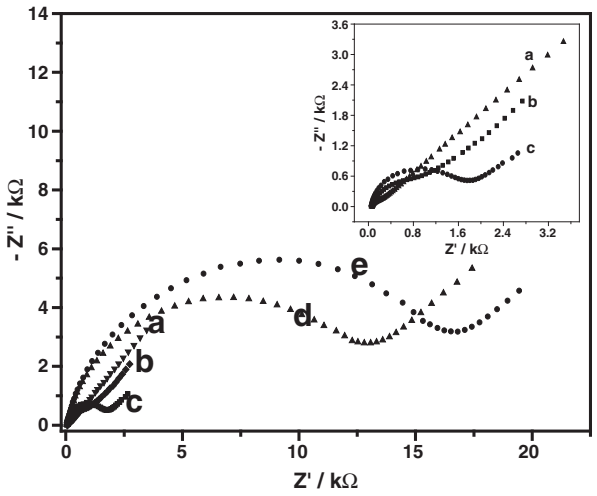

Fig. 2. EIS of bare GCE (a); chitosan–CNi/GCE (b); chitosan/GCE (c); tyrosinase–chitosan–CNi/GCE (d) and chitosan–tyrosinase/GCE (e) in 0.5 mM $Fe(CN)_6^{3-/4-}$-containing 0.5 M KNO_3 solution. The frequency range: 0.05 Hz to 1 00 kHz. Inset: close-up of plots (a), (b) and (c).

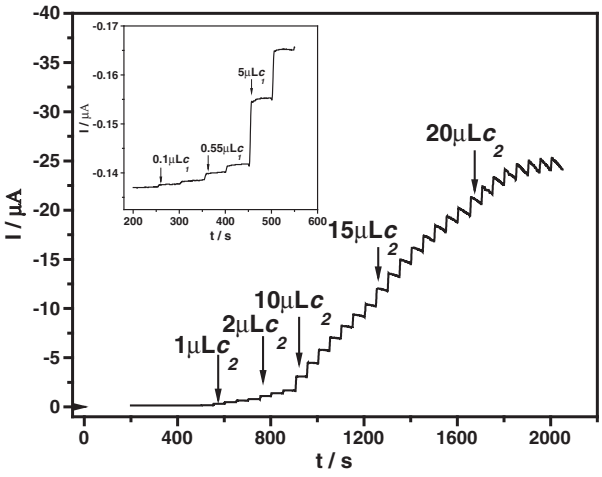

Fig. 4. Typical current-time response curve of tyrosinase–chitosan–CNi/GCE upon successive additions of catechol with different concentrations into the stirred 0.1 M pH 6.5 PBS. Inset: the magnification of the first six steps. Catechol concentrations: (c_1) 10.00 μM , (c_2) 1.00 mM. Applied potential: −0.2 V vs. SCE.

resistance of chitosan (c) was larger than that of CNi–chitosan film (b). Such phenomenon might be caused by the conductivity of CNi. Similar result was observed on tyrosinase–chitosan–CNi (d) and tyrosinase–chitosan (e) films. In addition, (d) and (e) showed obvious increase impedance indicating the successful immobilization of tyrosinase.

3.2. Electrochemical response of catechol at tyrosinase–chitosan–CNi/GCE

Fig. 3 displays the typical cyclic voltammograms of catechol in pH 6.5 PBS at bare GCE (a); chitosan–CNi/GCE (b); tyrosinase–chitosan/GCE (c) and tyrosinase–chitosan–CNi/GCE (d). As demonstrated, there was no obvious redox peak in the potential range of −0.4–0.2 V on the modified electrodes without tyrosinase. However, a significant increase of the reduction peak was observed with tyrosinase–chitosan/GCE and tyrosinase–chitosan–CNi/GCE, which was due to the reduction of *o*-quinone produced from the enzymatic reaction on the electrode surface [21]. The reduction peak current of catechol at tyrosinase–chitosan–CNi/GCE was higher, indicating that CNi played an important role in enhancing the electron transfer between *o*-quinone and the electrode surface[22].

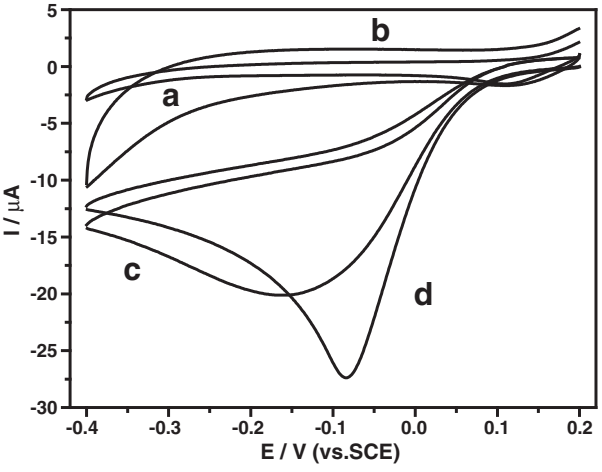

Fig. 3. Cyclic voltammograms of 74 μM catechol in PBS (pH 6.5), on a bare GCE (a); chitosan–CNi/GCE (b); tyrosinase–chitosan/GCE (c) and tyrosinase–chitosan–CNi/GCE (d). Scan rate: 100 mV s^{-1}.

3.3. Amperometric response of catechol

Fig. 4 illustrates a typical steady-state current–time curve for detecting catechol at tyrosinase–chitosan–CNi/GCE under the optimum conditions. When the modified electrode reached a steady baseline in blank PBS, catechol was added into the solution causing obvious current response, which was a result of the electrochemical reduction of *o*-quinone generated from the enzymatic oxidation of catechol [22]. The cathodic current reached 95% of steady-state current within 8 s. Fig. 5 exhibits the typical calibration curve of the biosensor to catechol. The linear range spanned from 0.25 nM to 27 μM, with the regression equation: $I_{pa}/\mu A = -0.30\ (\pm 0.066) - 0.51\ (\pm 0.0063)\ c(\text{catechol})/\mu M$. The detection limit was down to 0.083 nM at a signal to noise ratio of 3. The linear range was obviously wider than those tyrosinase electrodes based on polyaniline–ionic liquid-carbon nanofiber composite (0.40 nM–2.1 μM) [1], alumina sol–gel on Sonogel-Carbon transducer (0.10–30 μM) [9] and colloidal gold nanoparticles–graphite–teflon composite (0.01–8.0 μM) [23]. In addition, its sensitivity was 514 μA mM^{-1}, which was much higher than that reported based on ZnO nanorod

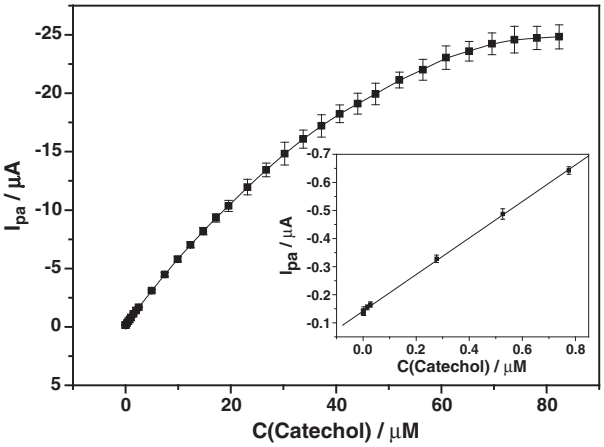

Fig. 5. Calibration curve of the biosensor as a function of the concentrations of catechol. Inset: the magnification of the first nine dots.

Table 1
Determination of catechol.

Samples (c/μM)	Added (c/μM)	Found ± S.D.[a] (c/μM)	Recoveries (%)
1.00	1.00	2.06 ± 0.07	106.0
1.00	4.00	5.14 ± 0.21	103.5
1.00	9.00	9.83 ± 0.29	98.1

[a] S.D.: standard deviation (n = 3).

(2.14 μA mM^{-1}) [24], multiwalled carbon nanotube–Nafion nanobiocomposites (346 μA mM^{-1}) [3] and single walled carbon nanotubes (355 μA mM^{-1}) [25]. According to Lineweaver–Burk equation [26], the apparent Michaelis Menten constant (K_M^{app}) of the enzyme biosensor was 59.31 μM for catechol. This value was significantly lower than that of tyrosinase immobilized in Fe_3O_4 nanoparticles–chitosan nanocomposite (96.9 μM) [27].

3.4. Reproducibility and stability of the biosensor

The reproducibility of the same tyrosinase–chitosan–CNi/GCE was examined by the detection of 5.0 μM catechol in 0.1 M PBS (pH 6.5) for ten successive determinations. A relative standard deviation (RSD) of 3.8% was obtained, which demonstrated that the modified electrode had good reproducibility. The fabrication reproducibility was investigated with five different modified electrodes, which were constructed independently with the same treatment. RSD value was 4.6%. The long-term stability of the biosensor was also estimated by measuring its response in 5.0 μM catechol after the electrode stored in dry state at 4 °C for 2 month. The biosensor retained 60.3% of its original response after 2 month. It demonstrated that CNi efficiently maintained the activity of tyrosinase to a large extent.

In order to demonstrate the potential application of the proposed method for the determination of catechol, a recovery test was carried out by adding known amounts of a catechol standard to the sample. As shown in Table 1, the recoveries obtained ranged from 98.1% to 106.0%, indicating that the biosensor was reliable for the quantification of catechol.

4. Conclusions

A novel tyrosinase biosensor based on chitosan–CNi nanobiocomposite film had been successfully prepared. The nanobiocomposite film was suitable for the immobilization of tyrosinase and the retention of its bioactivity to a large extent. CNi nanoparticles played an important role in enhancing the electron transfer between *o*-quinone and the electrode surface. The proposed biosensor could be used to detect trace phenolic compounds with remarkable properties such as fast response, broad linear range, low detection limit, high sensitivity, low K_M^{app} and accepted storage stability. With simple fabrication process and many benefiting characteristics, the chitosan–CNi nanobiocomposite film has great potential for development of other enzyme-based biosensors.

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