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Application of horseradish peroxidase modified nanostructured Au thin films for the amperometric detection of 4-chlorophenol

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ABSTRACT

A facile electrodeposition method was used to fabricate nanostructured Au thin films. Construction of the amperometric biosensors for 4-chlorophenol (4-CP) is based on the immobilization of horseradish peroxidase (HRP) on the Au thin films via a simple method. The as-prepared nano-Au films provide a favorable microenvironment for the immobilization of HRP and the retention of its activity. With H_2O_2 as oxidizing co-substrate, the HRP modified electrode displays high catalytic activity toward 4-CP. Besides, it has been demonstrated that the immobilization of HRP has a significant positive effect on the anti-fouling performance of the electrode material. Furthermore, the enzyme modified electrode was used as a bioelectrochemical sensor of 4-CP, exhibiting linear relationship in two different concentration ranges: from 2.5 to 40 μM and from 62.5 to 117.5 μM, with a detection limit of 0.39 μM (S/N = 3) at an applied potential of −0.55 V. The fast current response and good stability were obtained on the HRP modified electrode.

1. Introduction

Due to the high toxicity, persistence and bioaccumulation, a long-term threat to human health and ecological environment will be posed by chlorinated organic compounds if discharged into the environment [1,2]. And for chlorophenols (CPs), a higher toxicity and biorefractive ability will be resulted from a stable conjugation of chlorine atom and benzene rings. Therefore, CPs are listed as the priority pollutants by the U.S. Environmental Protection Agency (EPA, Toxic Substance Control Act, Washington, DC 1979). CPs, widely used as wood preservatives, herbicides and pesticides, constitute a major class of organic pollutants that contaminate the ecosystem and accumulate in the food chain. Monitoring of CPs is of particular importance in environmental control, remediation and food analysis.

Generally, the traditional detection method, based on spectrographic [3,4] and chromatographic analysis (gas chromatography–mass spectrometry [5,6] and high performance liquid chromatography [7,8]), has its intrinsic shortcomings, such as time consuming and incapability of real-time monitoring in real samples [9]. In contrast, because of their advantages in high sensitivity, fast response, easy operation and continuous on-line detection, electrochemical sensors have been widely used in clinical medicine, biochemistry and environmental monitoring. Successful application of the electrochemical detection of phenol derivates has been reported on boron-doped diamond electrodes and various modified electrodes [10–12]. Unfortunately, fouling is a common phenomenon at many electrode materials [13–16], which disturbs the application of electrochemical detection in phenolic wastewater.

Polyphenol oxidases (laccase [17,18], tyrosinase [19,20], etc.) have been attempted to construct the phenolic sensor. However, tyrosinase-based biosensors are limited to the monitoring of ortho-position phenolic compounds. Peroxidases can be used in biosensing devices for the determination of phenols based on their double displacement or "ping-pong" mechanism in which two substrates (i.e. hydrogen peroxide and the given phenolic compound) are involved [21]. Horseradish peroxidase (HRP) exhibits a relatively low specificity toward the electron-donor species (such as phenol derivatives) [22,23].

With the rapid development of nanotechnology, a great number of nanomaterials, especially the noble metal [24–27], have been introduced into the bioelectrochemical sensor. However, a severe fouling phenomenon will be caused on noble metal electrodes by oligomers and polymers as a consequence of chemical coupling of the corresponding phenoxy radicals during the electrooxidation of phenolic compounds [13,28]. So in order to enhance the resistance to fouling, in this paper HRP was immobilized on

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the nanostructured Au thin film, considering its excellent catalytic activity toward CPs [29,30]. The Au thin film was directly formed on the glassy carbon electrode surface with ease by a potential-step electrodeposition technique, which provides a better and more convenient boundary for enzyme immobilization than Au nanoparticles. The enzyme modified electrode displayed a remarkable anti-fouling ability for electrooxidation of 4-CP. Furthermore, as a bio-electrochemical sensor, the as-prepared enzyme electrode shows an excellent sensing performance toward 4-CP. The obtained results are helpful for the development of high-performance 4-CP biosensors.

2. Experimental

2.1. Reagents and materials

Horseradish peroxidase (HRP, EC 1.11.1.7, 225 U mg^{-1}) was purchased from Sigma Co. The chemical reagents were analytical grade and the aqueous solutions were prepared with ultrapure water (>18 MΩ cm). The phosphate buffer solution (PBS, 0.2 M, pH 6.8) was prepared by mixing NaH_2PO_4 and Na_2HPO_4 solutions.

2.2. Preparation of Au film modified electrode

Glassy carbon electrodes (GCEs, Φ = 5 mm) were ground with emery papers of decreasing particle sizes to no. 3500 finish at first, and then polished with 0.05 μm alumina slurry. Before use, the electrodes were cleaned ultrasonically in HNO_3 (1:1), washed with ultrapure water, and finally rinsed with absolute ethanol.

The Au film modified GCE (Au/GCE) was fabricated by double-potential step electrodeposition technique. In a typical case, the nanostructured Au thin films were directly formed on a clean GCE with two potential pulses in succession: a reducing nucleation pulse (+0.70 V) for 1 s and a growth pulse (−0.40 V) for 1800 s in a mixed solution of 0.5 M H_2SO_4 and 1 mM $HAuCl_4$. After thoroughly rinsing with ultrapure water, Au/GCE was successively placed in 0.5 M H_2SO_4 and 0.1 M PBS for carrying out the continuous potential cycling in the designated potential range until it gave steady cyclic voltammetric (CV) curves. The electrochemical active surface area (EASA) of the Au film was determined by the oxygen-adsorption method [31].

2.3. HRP immobilization

The HRP solution (4.0 mg mL^{-1}) was prepared by dissolving 4 mg HRP in the mixed solution of 800 μL of 0.1 M PBS and 200 μL of dimethyl sulfoxide and stored in refrigerator at 4 °C. The HRP modified electrode (HRP/Au/GCE) was prepared by dropping 5 μL of 4.0 mg mL^{-1} HRP solutions onto the Au/GCE and then dried in refrigerator at 4 °C. Finally, 5 μL of 0.1% Nafion was coated to form a protective film and also dried in refrigerator at 4 °C. Then the as-prepared enzyme modified electrode was rinsed and stored in refrigerator at 4 °C for use.

2.4. Apparatus and measurements

Surface morphologies of metallic architectures were observed by field emission scanning electron microscope (FESEM, Hitachi S-4800), and EDS analyses were recorded with an INCAX-sight energy dispersive X-ray spectrometer equipped on the FESEM.

All electrochemical measurements were performed with a CHI 760C electrochemical workstation (Shanghai CH Instruments, China) in a conventional three-electrode cell at room temperature (~15 °C). The modified GCE was selected as working electrode. A bright Pt plate (1.0 cm × 1.0 cm) and a saturated calomel electrode (SCE) served as the counter electrode and the reference electrode, respectively. All the potentials were referred to SCE. The reference electrode was led to the surface of the working electrode through a Luggin capillary. The amperometric response of 4-CP was recorded in 0.1 M deaerated PBS + 1 mM H_2O_2 mixed solutions at −0.55 V under strong stirring.

3. Results and discussion

3.1. Morphology characteristic of Au films before and after HRP immobilization

Nanostructured Au thin films can be directly deposited onto the surface of GCEs by double-potential step electrochemical techniques and the measured SEM image of Au films is shown in Fig. 1(a). The Au film seems to be constructed by a large quantity of nanoscale Au "islands". The biocompatible microenvironment of Au films is very suitable for enzyme immobilization. After HRP immobilization, the morphology of Au film has little change except for an ill-defined periphery of Au "islands", as seen in Fig. 1(c), which is perhaps caused by the poor conductivity of HRP. After 30 continuous CV cycles in the mixed solution of 0.1 M PBS + 1 mM H_2O_2 + 0.25 mM 4-CP, the structural features of Au films without and with HRP immobilization were shown in Fig. 1(b) and (d), respectively. The adhesion phenomenon of the unmodified Au film is serious (as shown in Fig. 1(b)), which is attributed to the deposition of an amorphous porous film originating from the polymerization of phenoxyl radicals during the electrooxidation of 4-CP on the Au film surface [13]. However, the HRP immobilization has a significant positive effect on the anti-fouling performance of the electrode material. As compared with Fig. 1(a), almost no change was observed on the surface structure of HRP modified electrodes after 30 CV cycles under the same test conditions (in Fig. 1(d)). The SEM results were also demonstrated by the following CV test.

3.2. Electrochemical behavior of HRP/Au/GCE

3.2.1. Inhibitory effect of the immobilized HRP on non-enzymatic redox reactions

Considering that the change of interface state usually has an effect on the electron transfer at the electrode/solution interface, the electrochemical redox probe is used to determine the influence degree. The effect of HRP immobilization on the electron transfer was investigated by the CV method using the p-benzoquinone/hydroquinone (BQ/HQ) couple as electrochemical redox probe (as shown in Fig. 2(a)). The potential difference ($\Delta E_p = E_{pa} - E_{pc}$) was 102 mV after HRP modification while only 62 mV was obtained for Au/GCE. Obviously, the immobilization of HRP leads to a peak-to-peak separation indicating the deviation from the reversibility for BQ/HQ redox reaction. The greatly diminished current response and enlarged ΔE_p indicate that an electron transfer barrier is introduced into the interface, which results from the retarding interfacial electron transfer kinetics [32,33].

3.2.2. Direct electrochemistry of HRP/Au/GCE

Though an inhibition effect on the electron transfer of non-enzyme mediated redox reactions at the electrode/solution interface will be caused by the immobilization of HRP, the direct electrochemical behavior still can be observed on the enzyme electrode. The cyclic voltammograms (CVs) of Au/GCE and HRP/Au/GCE in 0.1 M deaerated PBS are shown in Fig. 2(b). As compared with Au/GCE, a pair of redox peaks appear in the CV of HRP/Au/GCE, which should be attributed to the direct electron transfer involved in the redox process of HRP-Fe(II) and HRP-Fe(III) (HRP-Fe(III) + e^- + H^+ ↔ HRP-Fe(II)) [34,35]. The CV of HRP/Au/GCE exhibits an anodic peak at −0.366 V and a cathodic peak at 0.001 V at a scan rate of 50 mV s^{-1}. It is obvious that the as-prepared Au thin