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HIGHLY SENSITIVE CATALASE ELECTRODE BASED ON POLYPYRROLE FILMS WITH MICROCONTAINERS*

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Abstract Highly sensitive catalase electrodes for sensing hydrogen peroxide have been fabricated based on polypyrrole films with microcontainers. The microcontainers have a cup-like morphology and are arranged in a density of 4000 units cm^{-2}. Catalase was immobilized into the polypyrrole films with microcontainers (Ppy-mc), which were coated on a Pt substrate electrode. The catalase/Ppy-mc/Pt electrode showed linear response to hydrogen peroxide in the range of 0–18 mmol/L at a potential of −0.3 V (versus SCE). Its sensitivity was measured to be approximately 3.64 μA $(mmol/L)^{-1}$ cm^{-2}, which is about two times that of the electrode fabricated from a flat Ppy film (catalase/Ppy-flat/Pt electrode). The electrode is highly selective for hydrogen peroxide and its sensitivity is interfered by potential interferents such as ascorbic acid, urea and fructose. Furthermore, such catalase electrodes showed long-term storage stability of 15 days under dry conditions at 4°C.

Keywords: Microstructured polypyrrole; Catalase; Electropolymerization; Sensors; Hydrogen peroxide.

INTRODUCTION

It is important to determine hydrogen peroxide in food and environment, since very low level of hydrogen peroxide can damage the mammalian cells[1]. The conventional methods for detecting hydrogen peroxide including amperometry[2] and chemiluminometry[3] need laborious chemical reactions and long time. Researchers have been encouraged to design various hydrogen peroxide sensors to make more practical and reliable tests[4–8] using different enzymes. The most popular enzyme used in hydrogen peroxide biosensors is catalase[9].

As an effective way for fabricating hydrogen peroxide biosensors, entrapment of catalase in conducting polymer matrices is important. Polypyrrole (Ppy) is an intrinsically conducting polymer with many interesting properties such as high conductivity, good thermal and environmental stability, and good biocompatibility. Thus, various biosensors based on Ppy films have been fabricated[10, 11]. However, most of these attempts suffered from poor diffusion rate and low sensitivity. To overcome these difficulties, template guide synthesized polypyrrole nanotube arrays were used as the containers of enzyme, which improved the sensor's sensitivity greatly[12].

Recently, we synthesized polypyrrole microstructures with morphology like bowls, cups and bottles by using self-assembled gas bubbles as template[13–16]. The specific surface area of the Ppy film with microcontainers was much higher than that of a flat Ppy film. In this paper, we report the applications of these microstructured films for the fabrication of highly sensitive hydrogen peroxide sensor.

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EXPERIMENTAL

Reagents

(–)-Camphorsulfonic acid (98%, Fluka) and catalase (2000–5000 U/mg, Sigma) were used as received. Pyrrole (99%, Hongyu Chemical Company of Beijing Institute of Technology) was distilled prior to use. Hydrogen peroxide (30%, Beijing Chemical Factory) was of analytical grade. Ascorbic acid (Beijing Xizhong Chemical Factory), urea, Na_2HPO_4 and NaH_2PO_4 (Beijing Yili Fine Chemical Factory) were all of analytical grade and used as received. Fructose (Tianjin Bodi Chemical Limited Company) was a biological reagent. An aqueous solution of 0.5 mol/L pyrrole and 0.6 mol/L (–)-camphorsulfonic acid was used as the electrolyte for electrochemical polymerization. The flat Ppy film was grown potentiostatically at 0.75 V (versus saturated calomel electrode (SCE)) on a Pt working electrode for 60 s.

Instruments

All electrochemical experiments were carried out at room temperature in a conventional one-compartment cell (5 cm^3) connected to a potentiostat (EG&G, model 263A) with three electrodes. Two Pt electrodes with surface area of 1.0 cm^2 each were used as the working and the counter-electrodes. The Pt electrodes were mechanically polished with 0.3 μm alumina polishing cloth, then thoroughly rinsed with distilled water and dried in air. An SCE was used as a reference electrode. The scanning electron microphotographs (SEMs) were taken by using a KYKY2800 scanning electron microscope (Beijing Scientific Instrument Company, China). An aqueous solution of 0.5 mol/L pyrrole and 0.6 mol/L (–)-camphorsulfonic acid was used as the electrolyte for electrochemical polymerization. The flat Ppy film was grown potentiostatically at 0.75 V (versus SCE) on a Pt working electrode for 60 s.

Fabrication of Catalase Electrodes

In order to grow Ppy film with microcontainers, first the solution was pretreated by cyclic voltammetric scanning over the potential range from 0 to 1.4 V at the potential scan rate of 20 mV/s for two cycles to produce enough suspended gas bubbles in the solution. Then a Pt sheet was inserted into the solution carefully and a large amount of tiny bubbles were assembled on the working electrode surface. The Ppy films with microcontainers were also deposited on Pt electrode potentiostatically at 0.75 V (versus SCE) for 60 s. After polymerization, the electrodes were rinsed with distilled water repeatedly and then pretreated in 0.1 mol/L phosphate buffer solution by cyclic voltammetric scanning over the potential range from –0.6 to +0.6 V at the potential scan rate of 50 mV/s for 20 cycles. Successively, the Ppy films on the electrodes were dedoped in the same medium at a potential of –0.6 V for 600 s.

Catalase was dissolved in 0.01 mol/L phosphate buffer solution to prepare 4 mg/mL catalase solution. Then the dedoped Ppy films were doped in the catalase solution at the potential of –0.3 V for 20 min[17]. After that, a 0.1 mol/L phosphate buffer was used to wash the electrode to remove the loosely adsorbed catalase. All the procedures described above were carried out at 25°C. The obtained catalase electrodes were preserved in 0.1 mol/L phosphate buffer solution (pH = 7) at 4°C. The electrodes fabricated from flat and microstructured Ppy films are denoted as catalase/Ppy-flat/Pt and catalase/Ppy-mc/Pt electrode, respectively.

Measurements of Amperometric Responses of Catalase Electrodes

Cyclic voltammograms were performed in 0.10 mol/L phosphate buffer solution in the presence/of absence hydrogen peroxide. The catalase electrode was scanned for several cycles in buffer solution with gentle stirring before the injection of hydrogen peroxide. The geometric surface area for each electrode was the same and the potential scan rate was 50 mV/s. Then 5 mL 0.1 mol/L phosphate buffer solution (pH 7) was used as sensing solution. The catalase electrode was maintained at 25°C at a constant polarization potential of –0.3 V (versus SCE) in an air-saturated sensing solution and a stable background current was obtained after 20 min. Then a quantitative amount of hydrogen peroxide solution was injected into the sensing solution by a micro-injector successively to make the concentration of hydrogen peroxide increase for 1 mmol/L each time in the range from 0 to 20 mmol/L. The solution was briefly mixed and a steady-state current was achieved after 4–6 min. In order to investigate the detection limit, a low concentration of hydrogen peroxide from 0.001 to 0.030 mmol/L was also prepared.

Measurements of the Influences of Interfering Reagents

The current response of a catalase electrode on 1 mmol/L hydrogen peroxide at −0.3 V in 0.10 mol/L phosphate buffer (pH = 7) was measured first, then each of the several potential interfering reagents, such as ascorbic acid, fructose and urea, was added into the sensing solution, respectively. The concentration of each interfering reagent was in biological level (0.5 mmol/L).

RESULTS AND DISCUSSION

Morphology of the Ppy Films

Figure 1 shows the SEM images of a flat Ppy film and a Ppy film with microcontainers. The surface of a flat Ppy film (Figs. 1a and 1b) is rough in nanometer scale, which supplies a large specific surface area to support the catalase. In Fig. 1(c), the Ppy film with microcontainers has micro-cups standing upright on the electrode surface and the micro-cups are arranged fairly well in a high density, approximately 4000 units cm^{-2}. Most of the micro-cups have calibers of approximately 80 μm and height of approximately 100 μm. It is shown that the Pt substrate remained uncovered at the bottom of each micro-cup. The surface of the Ppy film with microcontainers was much rougher than that of the flat Ppy film, which contributes more specific areas of the films. Furthermore, the aggregates of Ppy were loosely packed in the film, which reduces the diffusion barrier for dopants and substrate molecules.

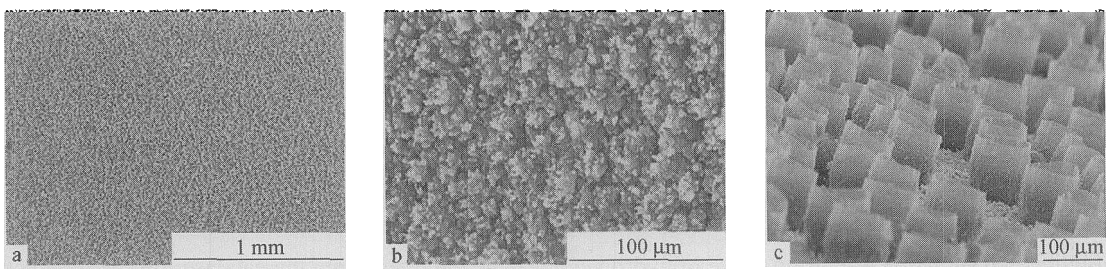

Fig. 1 Scanning electron microphotographs of polypyrrole films
A flat Ppy film (a, b) and a Ppy film with microcontainers (c) were grown potentiostatically at 0.75 V (versus SCE) on Pt electrodes for 60 s.

Effect of Microstructures of Ppy Films on the Current Responses of Catalase Electrodes

Figure 2 illustrates the cyclic voltammograms of a catalase/flat-Ppy/Pt electrode in 0.10 mol/L phosphate buffer

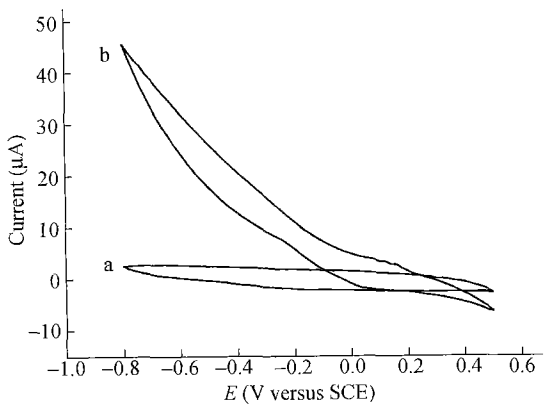

Fig. 2 Cyclic voltammograms of a catalase/flat-Ppy/Pt electrode in 0.10 mol/L phosphate buffer solution (pH = 7) at a potential scan rate of 50 mV/s: (a) without hydrogen peroxide and (b) with 10 mmol/L hydrogen peroxide
The electrode was scanned for 1 cycle in the buffer solution with gentle stirring before the injection of hydrogen peroxide in curve (b).

solution without hydrogen peroxide in Fig. 2(a) or with 10 mmol/L hydrogen peroxide in Fig. 2(b). It could clearly be seen from this figure that the electrochemical response of the electrode in the medium without hydrogen peroxide is weak. In contrast, response to hydrogen peroxide occurs at the potentials lower than 0 V with a strong cathodic current as shown in Fig. 2(b). This proves that the adsorbed catalase maintains its biological activity.

The current-time curves shown in Figs. 3(a) and 3(b) indicate that the catalase/Ppy-mc/Pt electrode has rapid response and high sensitivity to hydrogen peroxide. After the injection of 1 mmol/L hydrogen peroxide each time, the anodic current increased dramatically and reached a steady state within 2 min at a polarization potential of –0.3 V. Since the catalase-catalyzed reaction is kinetically a first-order reaction with respect to the substrate[18], the current related to decomposition of hydrogen peroxide will be proportional to the concentration of hydrogen peroxide.

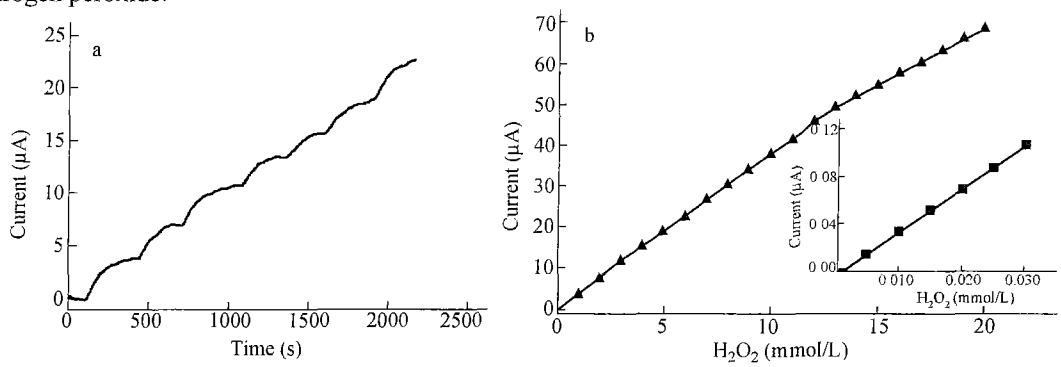

Fig. 3 (a) Typical current-time curve for a catalase/Ppy-mc/Pt electrode in a 0.1 mol/L phosphate buffer (pH = 7) at 25°C at –0.3 V (versus SCE) and (b) calibration curves for hydrogen peroxide using the catalase/Ppy-mc/Pt electrode The concentration increase of hydrogen peroxide in each step is 1 mmol/L.

Figure 3(b) shows a calibration curve of hydrogen peroxide obtained using the catalase/Ppy-mc/Pt electrode. Measurement conditions were the same as in Fig. 3(a). Inset graph is an enlargement of low hydrogen peroxide concentration region. The response current was linear in the concentration range from 0 to 18 mmol/L (correlation coefficient $R = 0.998$) with a detection limit of 5×10^{-3} mmol/L. The sensitivity (slope of the linear range) was estimated to be 3.64 μA $(\text{mmol/L})^{-1}$ cm^{-2}.

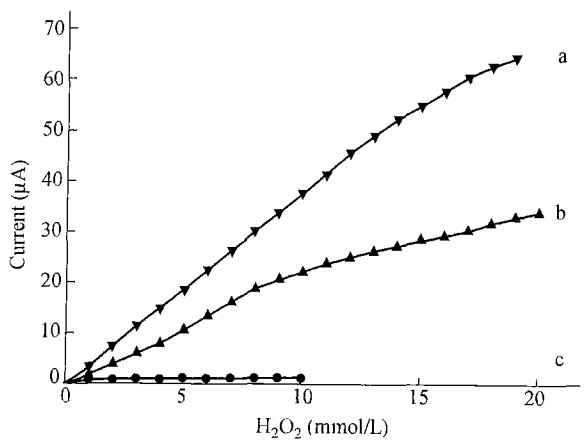

Fig. 4 Calibration curves for different electrode responses to hydrogen peroxide in a 0.1 mol/L phosphate buffer (pH = 7) at 25°C at –0.3 V (versus SEC)
(a) catalase/Ppy-mc/Pt electrode; (b) catalase/Ppy-flat/Pt electrode; (c) Ppy-flat/Pt electrode

Figure 4 shows the plots of amperometric responses of the catalase electrodes versus the hydrogen peroxide concentrations at 25°C. The sensitivity of a sensing electrode is represented by current response/(concentration of hydrogen peroxide × initial area of the electrode). The sensitivities were approximately 3.64 μA $(mmol/L)^{-1}$ cm^{-2} for the catalase /Ppy-mc/Pt electrode (curve a), and approximately 1.76 μA $(mmol/L)^{-1}$ cm^{-2} for the catalase /Ppy-flat/Pt electrode (curve b), respectively. With the increase of hydrogen peroxide concentration, the sensitivity gradually decreased. The curve shows excellent linearity: the linearity ranges from 0 to 18 mmol/L (correlation coefficient R = 0.998) for catalase/Ppy-mc/Pt electrode and 0 to 9 mmol/L (correlation coefficient R = 0.985) for the catalase/Ppy-flat/Pt electrode. As a comparison, a negligible response is observed at Ppy/Pt electrode in the 0.1 mol/L phosphate buffer (curve c).

The total charge amounts consumed to grow the flat film and the Ppy film with microcontainers on Pt electrodes were nearly the same as described in the experimental section, the response current of the electrode based on the Ppy film with microcontainers is 2.07 times that of the electrode with a flat film. This is mainly due to the fact that the specific surface area of the microstructured Ppy film is much larger than that of a flat film as demonstrated in Fig. 5. Furthermore, the bottom of each micro-cup was not coated with Ppy film and the product in the inner wall of the micro-cups could achieve the substrate Pt electrode easily. The diffusion barrier for product in the Ppy film with microcontainers was much lower than that in a flat Ppy film, which is also an important reason for improved sensitivity.

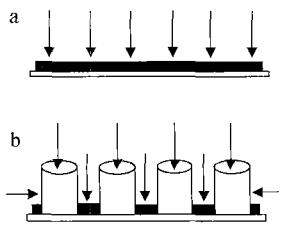

Fig. 5 Schematic diagram of (a) Ppy thin film and (b) microstructured Ppy-film on Pt substrate exposed to hydrogen peroxide molecules (arrows) Compared to a thin film, microstructures have a much higher fraction of exposed surface areas, and hydrogen peroxide molecules can diffuse to the Pt electrode with lower diffusion barrier for detection.

Currents Caused by Interferential Reagents and Storage Time of the Catalase/Ppy-mc/Pt Electrode

The influences of potential interferents such as ascorbic acid, urea and fructose on the catalase/Ppy-mc/Pt based sensor were investigated. The results demonstrated that interference levels were low or negligible and the catalase/Ppy-mc/Pt electrode showed improved selectivity as shown in Fig. 6. The reason may be that catalase

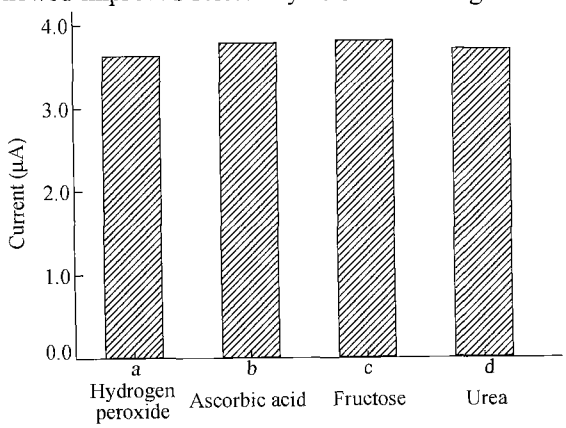

Fig. 6 (a) Current response of a catalase/Ppy-mc/Pt electrode on 1 mmol/L hydrogen peroxide in 0.1 mol/L phosphate buffer (pH = 7) at 25°C at −0.3 V (versus SEC), and (b, c, d) the current responses of the same electrode on 1 mmol/L hydrogen peroxide in 0.10 mol/L buffer solution (pH = 7) after adding biological level of 0.5 mmol/L ascorbic acid, 0.5 mmol/L fructose and 0.5 mmol/L urea, respectively

has an absolute specificity on hydrogen peroxide. And a high reduction potential of –0.3 V (versus SEC) was used to detect hydrogen peroxide and at this potential the interfering reagents cannot be reduced.

Furthermore, the catalase/Ppy-mc/Pt electrode still retains its stability after 15 days storage under dry conditions at 4°C and shows excellent activity. The current response to 1 mmol/L of H_2O_2 was about 95% of the original activity after 14 days. This excellent storage stability may be attributed to a stable complex formation of the catalase in the Ppy-mc layer. In contrast, when the electrode was kept at 4°C in 0.1 mol/L phosphate buffer, the response decreased to about 75% of the original value after 10 days.

CONCLUSIONS

Polypyrrole films with microcontainers such as micro-cups can be used to immobilize catalase. The catalase electrodes fabricated from polypyrrole films with microcontainers have a much higher sensitivity than that based on flat Ppy films. The catalase/Ppy-mc/Pt electrode shows linear response to hydrogen peroxide from 0 to 18 mmol/L at a potential of –0.3 V (versus SEC). The sensitivity is approximately 3.64 μA $(mmol/L)^{-1}$ cm^{-2}. The electrode has a high selectivity for hydrogen peroxide and good environmental stability.

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