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Fabrication of Prussian Blue modified ultramicroelectrode for GOD imaging using scanning electrochemical microscopy

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Abstract

A Prussian Blue (PB) film modified disk ultramicroelectrode (UME) was fabricated by electrochemical deposition technique on a Pt-disk UME. The electrocatalytical reductions of hydrogen peroxide derived from glucose oxidase (GOD) on this modified UME were investigated. The enzymatic biochemical reactivity was imaged by scanning electrochemical microscopy (SECM) utilizing the PB film modified UME. It is evident that sensitivity and spatial resolution for hydrogen peroxide measurement were improved obviously. SECM images obtained clearly revealed the concentration profile of the reaction products around the enzymes. The PB film modified microelectrode is in the nature of simple preparation, high catalytic activity on hydrogen peroxide and substrate selectivity for SECM etc.

Keywords: Modified ultramicroelectrode; Prussian Blue; Hydrogen peroxide; Enzyme; SECM

1. Introduction

The electrochemical detection of substances released by tiny living things (e.g. enzymes, cells) proceeding from enzymatic or cellular activities or upon stimulation with a specific chemical agent has been receiving numerous interests. However, all these require the positioning of suitable and selective sensors or detectors close to the substrate population. The ultramicroelectrode has always been attached to great interests and investments in electrochemical micro-analysis due to its excellent characteristics [1].

It has been demonstrated that SECM can provide sensitive techniques for determining the local activities and viabilities of immobilized enzymes [2–3], for its well applicability to detect trace amount of molecules in extremely little volumes. There are many applications of SECM in characterizing various types of samples, especially in biological systems [4]. Imaging of oxidation–reduction processes in single cells, such as metabolic regulations [5–6], especially photosynthetic [7–9] or respiratory activities [9,13], is of great interests because enzymatic redox reactions are essential to many cellular functions "working".

As the probe in SECM, UME with micro- or even submicrometer tip diameters are employed. In general, a micrometer-sized UME is positioned in close proximity to a living cell substrate and used to oxidize (or reduce) the molecules ejected from the cells. Topographic images are obtained by moving the UME across the sample surface, usually at a constant height, and monitoring the amperometric or potentiometric tip responses as the activities of living cells [10–13]. However, the probes commonly used in SECM are mostly bare metal electrodes (e.g. platinum wires) displaying inevitable drawbacks in some respects and need improvement. For instance, in order to detect sensitively and selectively the nitric oxide (NO) which was released from endothelial cells, a modified and reformative Pt-disk microelectrode was employed for SECM detection the release of NO [14–15].

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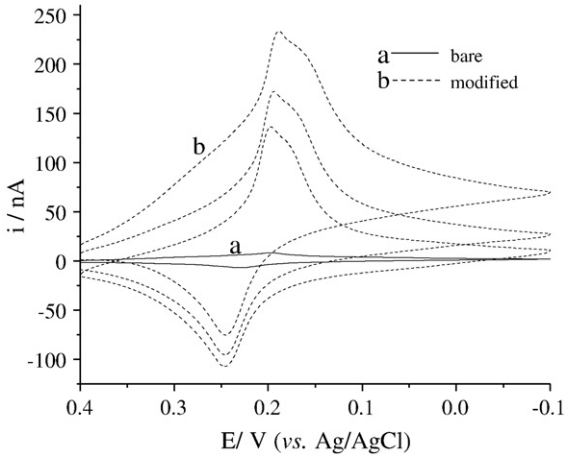

Fig. 1. Cyclic voltammetry curves of hydrogen peroxide in KCl solution. Solid line (a) was recorded with unmodified Pt UME while dotted lines (b) with PB film modified UME. Cross dotted lines obtained at different concentrations of hydrogen peroxide. Hydrochloric acid was used to adjust the pH value at the range of 5.0–7.0 and scanning potential range was −0.1 V–0.4 V.

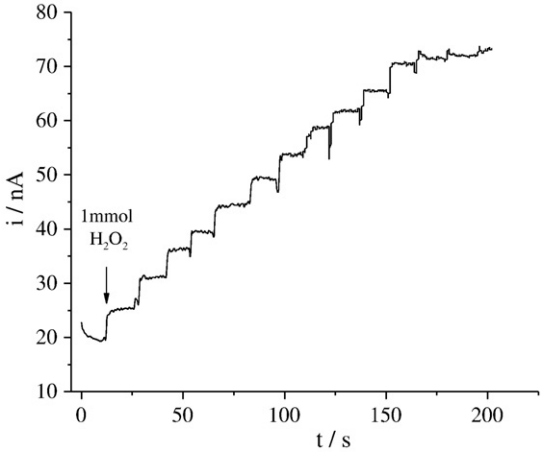

Fig. 3. Amperometric *i–t* response curve of hydrogen peroxide on PB film modified microelectrode. Each climbing step means adding 1 mmol of hydrogen peroxide successively.

Prussian Blue film modified electrodes come forth as early as in 1978 for its excellent electrochemical reversibility, high stability and simple preparation and so on. Due to PB film's particular electrocatalytical reduction performances on hydrogen peroxide, it's seemed as "artificial peroxidase" and can be used to functionalize biosensor combined with various enzymes, such as in detecting glucose [16], cholesterol [17], glutamate [18] and even herbicides [19], etc.

However, the application of PB film modified microelectrodes in SECM measurements may have not been reported to the best of our knowledge up to now. The present work proposed PB film modified microelectrode to be a probe of SECM. The electrocatalytical reduction performances of PB film modified microelectrode on hydrogen peroxide were inspected. The enzymatic reactivities were also mapped by SECM. The aforementioned micro-sensors are more selective and sensitive compared with the unmodified ones and offer greater promises for hydrogen peroxide determination than bare Pt microelectrodes.

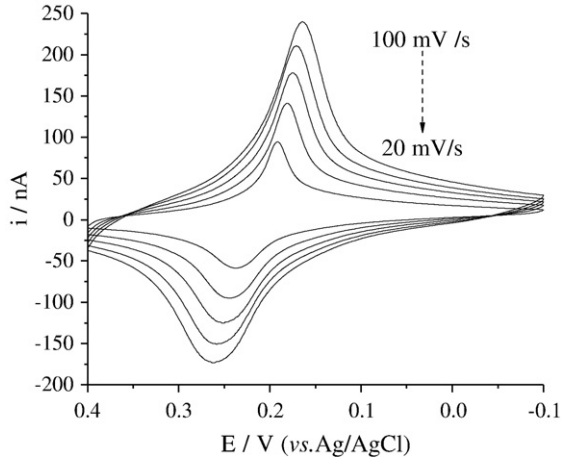

Fig. 2. Voltammetric curves with various scanning rates. From the top down, the scanning rates are 0.1 V/s, 0.08 V/s, 0.06v/s, 0.04 V/s and 0.02 V/s respectively.

2. Experimental

2.1. Chemicals and apparatus

Glucose and glucose oxidase (GOD) were purchased from Toyobo Company (Japan). All the other inorganic chemicals were analytical reagent grade and used as received. Deionized water (18.2MΩ cm, Millipore Synergy 185) was used in experiments. All experiments and measurements were carried out at room temperature 25 ± 1°C.

CHI660B electrochemistry workstation (Chenhua Instrument Company, Shanghai, China) was used to perform cyclic voltammetry and PB deposition experiments. The electrochemical cell was a classical three-electrode setting with an Ag/AgCl as reference electrode, a platinum wire as counter-electrode and the Pt UME with a diameter of 10μm as working electrode; CHI900A scanning electrochemical microscopy (CH Instrument Company, TX, USA) was used to perform enzymatic bioactivity mapping which were carried out in a there-electrode configuration with the

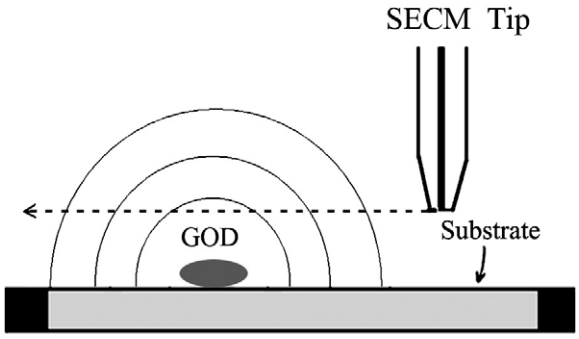

Fig. 4. Schematic illustration of activity image of GOD by SECM.

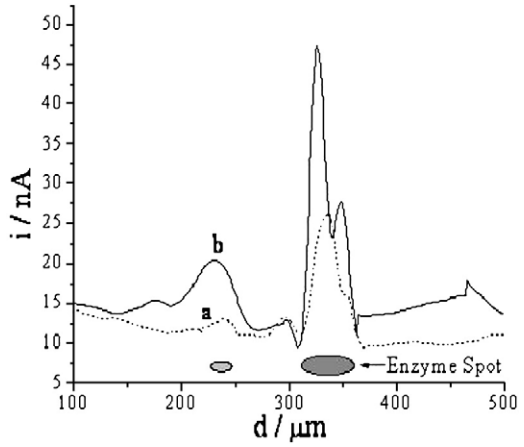

Fig. 5. The current comparison diagram of bare and PB film modified microelectrode. The cross sectional planes of SECM images across GOD spot producing H_2O_2 reduction currents with bare Pt microelectrode (dotted line a) and PB film modified microelectrode (solid line b).

PB film modified Pt UME as working electrode, an Ag/AgCl as reference electrode and a platinum wire as counter electrode.

2.2. Modification of the microelectrode

A Pt-disk UME (Φ = 10μm) surface was polished lightly with 0.05μm alumina powder on the chamois and bathed in HCl (1:1) and ethanol subsequently, and then it was ultrasonicated in deionized water for several minutes.

The PB film modified electrodes were normally constructed by the use of cyclic voltammetric method [20,21] and constant-potential electrodeposition [22]. Cyclic voltammetric method was adopted in order to electrodeposit PB film on the UME which was immersed in 2mmol/L $K_3[Fe(CN)_6]$ + 2mmol/L $FeCl_3$ + 0.1mol/L KCl mixed solution and carried out in a small beaker as the electrochemical cell until the voltammogram's steady-going. Thus the PB film modified disk ultramicroelectrode was fabricated by electrodeposition technique.

3. Results and discussion

3.1. Voltammetric characteristics of PB film modified UME

The PB film modified Pt UME was used to perform cyclic voltammetry in phosphate buffer solution (PBS) containing 0.1mol/L KCl in order to test the catalytical performances of PB film on hydrogen peroxide at a scan rate of 0.05V/s.

As manifested in Fig. 1, on the unmodified microelectrode no obvious oxidation–reduction reaction of hydrogen peroxide (curve a) occurred between − 0.1V and 0.4V for its apparently smooth and low-current value curve, which couldn't be competitive with the status on the PB film modified one for significant peaks of oxidation and reduction at about 0.24V and 0.19V respectively (curve b). Also from Fig. 1, the reduction peak currents amplified with the increase of hydrogen peroxide concentrations.

The cyclic voltammetric method was also adopted to record the oxidation–reduction currents of PB film modified UME in the PBS containing 2mmol/L H_2O_2 and 0.1mol/L KCl at different scanning rates (20mV/s–100mV/s). The result was shown in Fig. 2. It could be found that both oxidation and reduction peak currents were in proportion to the square root of scanning rates from experimental data, which indicated that the reaction of hydrogen peroxide on the PB film modified microelectrode was under the control of surface diffusion of hydrogen peroxide towards the modified microelectrode surface.

3.2. Amperometric response of PB film modified UME on hydrogen peroxide

In order to examine the sensitivity of amperometric response of the PB film modified UME on hydrogen peroxide, amperometric i–t curve was recorded and the result was shown in Fig. 3. When small droplets of hydrogen peroxide of 1mmol were added into the electrochemical cell, the amperometric responses increased immediately and also achieved the steady values at the same moment which indicated that the PB film has fast response to hydrogen

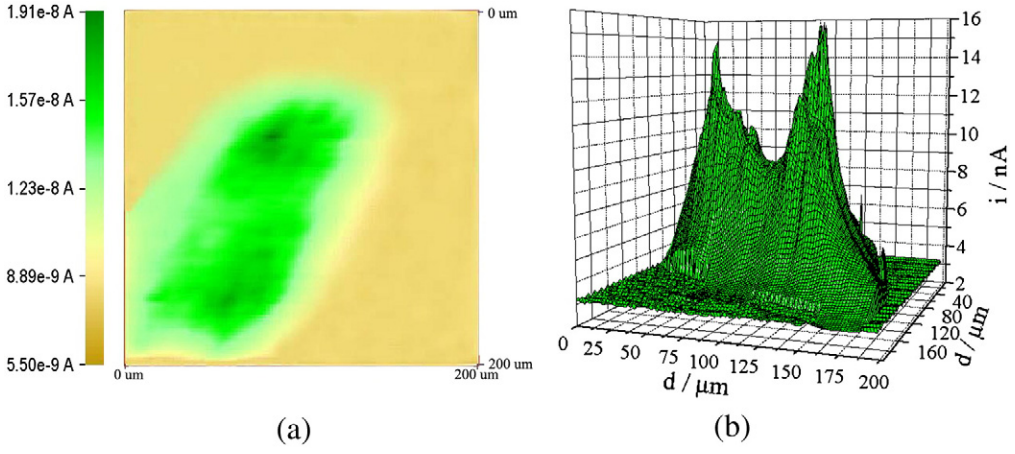

Fig. 6. SECM images of GOD spot activity (a) and its topographic graph (b). Two peaks may imply a significant quantum of scatter for two peaks that were overlapped.

peroxide. It is the evidence that the PB film has a high sensitivity on electrocatalysis of hydrogen peroxide.

Furthermore, the present conclusion educed that there was a linear correlation between the peak currents and the concentrations of hydrogen peroxide within the range of 1×10^{-5}–4×10^{-4}mol/L. Therefore, the PB film modified UME can be used to measure low concentrations of hydrogen peroxide in the environment.

3.3. The optimization of supporting electrolyte and pH value

The voltammetric characteristics of PB film modified UME in PBS containing different types of electrolytes of KCl, KNO_3, NaCl, NaAc (c = 0.1mol/L) were studied respectively. According to the ionic effect test, the highest peak current occurred in KCl solution.

The trend of the dependence of pH on peak current was investigated in detail from 1.0 to 7.5 in 0.1mol/L KCl solution. The experimental results showed that the maximum amperometric response of hydrogen peroxide on the PB film modified microelectrode was correspondingly dependent of pH over a range of 5.0–7.0. Thus the experimental supporting electrolyte and acidity were visualized.

3.4. Reproducibility and stability of the PB modified UME

The fabrication reproducibility of the PB film modified microsensor was estimated by determining the identical concentrations of hydrogen peroxide solutions in the same condition. The relative standard deviation (RSD) of the amperometric responses was 2.50% (n = 7).

The stability was investigated over a three and six hours' period respectively by continuously detecting the identical hydrogen peroxide solution. The amperometric response reduced to 97.2% and 93.4% respectively. It was also tested by cyclic voltammetry periodically in hydrogen peroxide solution and the reductive current slightly decreased after 250 scans (approximately reducing to 91.8%). Hence the PB film modified micro-sensor kept good amperometric response which revealed good stability.

4. Studies on GOD by SECM

4.1. Probe scan curves by SECM

A very small droplet of glucose oxidase solution was spotted onto the polyethylene substrate with a glass capillary and immobilized with 2% glutaraldehyde solution which was carried out with the help of microscope. According to the references [23,24] reported previously, the substrate of GOD spot was bathed in a mixed solution of 0.1mol/L PBS (pH = 6.8) containing 0.1mol/L KCl and 25mmol/L glucose. The tip was positioned in close proximity to the sample zone by means of the feedback mode (poised to 0.1V *vs.* Ag/AgCl). To invoke the generation-collector mode, the probe was then biased at 0.05V (*vs.* Ag/AgCl) for amperometric detection of hydrogen peroxide produced at the site of the immobilized enzymatic activity at a 1μm/s scan rate. Schematic illustration of GOD activity mapping by SECM was shown in Fig. 4.

While the GOD spot was scanned with the PB film modified tip again by means of the method adopted above, the reduction current derived from enzymatic hydrogen peroxide increased significantly which was shown in Fig. 5. There was a considerable amount of scatter for two peaks overlapped with the modified microelectrode than bare microelectrode. Thus it can be demonstrated that the sensitivity of detecting trace hydrogen peroxide produced by GOD with the presence of PB film increased markedly compared with that unmodified microelectrode. It is evident that spatial resolution of SECM for hydrogen peroxide measurements was improved obviously.

4.2. Imaging of GOD by SECM

According to the afore-cited method, a very small droplet of GOD solution was spotted onto the Pt substrate which was then immersed in a 0.1mol/L KCl and 0.1mol/L PBS (pH = 6.8) mixed solution also with 25mmol/L glucose. The immobilized enzymatic activity was imaged by the generation–collection mode of SECM with a PB film modified Pt UME (poised to 0.05V *vs.* Ag/AgCl) which acted as an amperometric probe to detect the reduction current of hydrogen peroxide. The SECM image obtained by an assisted constant-height mode (approximately 20μm above the substrate) at a scan rate of 1μm/s was shown in Fig. 6(a) and topographic graph in Fig. 6(b). It is apparent from Fig. 6 that when the PB film modified UME moving above the GOD zone where the concentration of hydrogen peroxide increased due to the enzymatic reaction, the reduction current was amplified.

5. Conclusions

It was demonstrated that the PB film modified UME could be used to detect trace hydrogen peroxide sensitively during the proportionately longer electrode's serviceable life. With the PB film modified disk ultramicroelectrode employed as a probe in SECM, the sensitivity, spatial resolution and image quality for the measurement of hydrogen peroxide produced by GOD were improved obviously compared with unmodified one. Thus, the PB film modified UME could be a convenient and powerful tool in SECM imaging.

Acknowledgments

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