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Hydrogen Peroxide Biosensor Based on the Direct Electrochemistry of Myoglobin Immobilized in Poly-3-Hydroxybutyrate Film

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Abstract: Direct electrochemistry of myoglobin (Mb) was observed in a stable film composed of a natural lipid polymer (poly-3-hydroxybutyrate) and Mb, the film of which was modified on a pyrolytic graphite electrode. The apparent formal potential of Mb was at about -260 mV in an acetate buffer solution with pH 5.0. Moreover, Mb in the polymer film exhibited catalytic activity towards the reduction of hydrogen peroxide (H_2O_2). Consequently, an unmediated biosensor for H_2O_2 was prepared with a linear range from 1.0×10^{-7} to 4.0×10^{-4} M.

Key words: Hydrogen peroxide, myoglobin, poly-3-hydroxybutyrate

INTRODUCTION

In nature, biomolecular superstructures containing enzymes or redox proteins are employed to shuttle electrons in many important life processes. Using electrochemical methods, we can make an in-depth research on these electron transfer pathways and the functions of the proteins and develop some useful devices such as biosensors. However, since the electron exchange between most enzymes or redox proteins and traditional electrode is usually prohibited, considerable research, including using electron-transfer mediators[1] and modified electrode surfaces[2], has been carried out to provide an electron-transfer compatible interface. Meanwhile, since many enzymes are bound onto or within lipid membranes in living cells, a new method has been brought forward, that is, to cast redox proteins into biomimetic films, such as lipids[3,4], surfactants[5,6] and polymer films[7,8], which are modified on electrode surface to achieve direct electron exchange between enzymes and electrodes. This method simplifies biodevices by removing the requirement for a chemical mediator[9]. Moreover, enzyme-coated electrodes can provide the basis for constructing biosensors, biomedical devices and enzymatic bioreactors that have wide applications in biotechnology[10].

We have recently reported that the electron transfer rate of hemoglobin can be enhanced after the protein is incorporated in poly-3-hydroxybutyrate (PHB)[11]. Since myoglobin (Mb) has a structural and functional similarity with hemoglobin, it is reasonable to investigate the electrochemical and electrocatalytic behavior of Mb. In this study, we report that Mb immobilized in PHB film shows greatly enhanced, quasi-reversible electron transfer and fine catalytic activity toward H_2O_2, which provides a model for constructing a third-generation H_2O_2 biosensor.

Experimental

Chemicals: Horse heart myoglobin (MW 17,800) and poly-3-hydroxybutyrate (MW 3,155) were obtained

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from Sigma. H_2O_2 (30% (w/v) solution, analytical grade) was from Nanjing Chemical Reagent Co. They were used without further purification. Other chemicals were all of analytical grade. All solutions were prepared by double distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of > 16 M cm^{-1} and stored in the refrigerator at the temperature of 4°C when not in use.

Preparation of Mb-PHB film: Press a basal plane Pyrolytic Graphite (PG) rod (geometric area: 5.45 mm^2) into a glass tube (with a diameter of 5 mm) and put epoxy resin at the glass/rod interface to fix it. Electrical contact was made by adhering a copper wire to the rod with the help of Wood alloy.

The substrate PG electrode was firstly polished on rough and fine sand papers and then with an alumina (particle size of about 0.05 μm)/water slurry on silk. Eventually, the electrode was thoroughly washed by ultrasonicating in both double distilled water and ethanol for about 5 min.

PHB suspension (1 mg mL^{-1}) was prepared by dispersing PHB in double distilled water with ultrasonication for about 45 min. Before preparing the films, the dispersion was ultrasonicated for another 10 min.

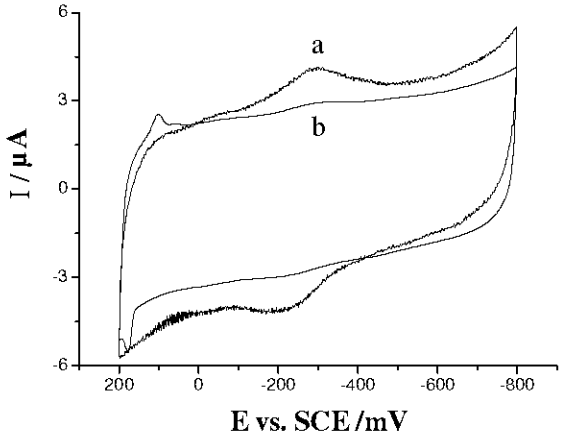

Fig. 1: Cyclic voltammograms at 200 mV s^{-1} in 0.1M acetate buffer with pH 5.0 for (a) Mb-PHB film, (b) PHB film.

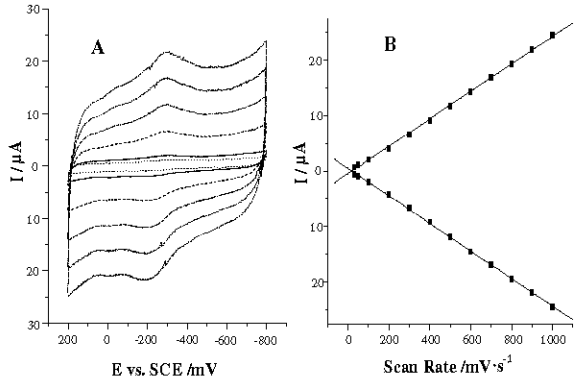

Fig. 2: A), Cyclic voltammograms of Mb-PHB/PG electrode in 0.1M acetate buffer of pH 5.0 at scan rates of 50, 100, 300, 500, 700, 900 mV s^{-1} (from inner to outer). B), effect of scan rate (v) on cathodic (P_c) and anodic (P_a) peak current (I) of Mb.

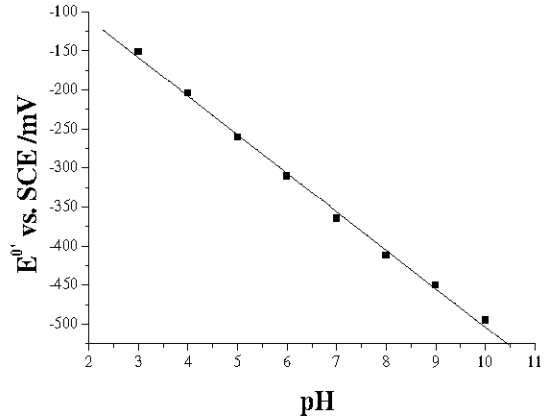

Fig. 3: The effect of pH value on the formal potential ($E^{0'}$) of Mb.

Typically, 10 L of the dispersion containing 5.2×10^{-5} M Mb and 0.5 mg mL^{-1} PHB was spread evenly onto PG electrodes for preparing Mb-PHB films.

The electrode surface was covered with an Eppendorf tube in the first two hours to prepare a uniform film and then dried in the air.

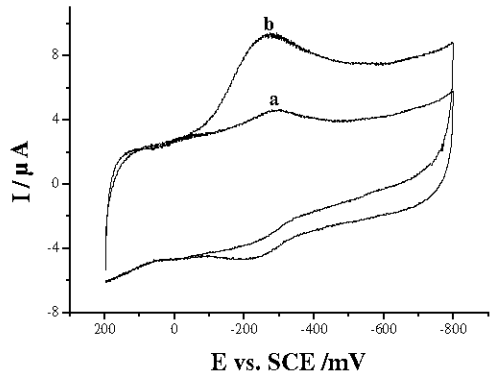

Fig. 4: Cyclic voltammograms obtained at a Mb-PHB modified PG electrode for a 0.1 M acetate buffer solution, pH 5.0, (a) before and (b) after the addition of 100 M H_2O_2 to the buffer solution.

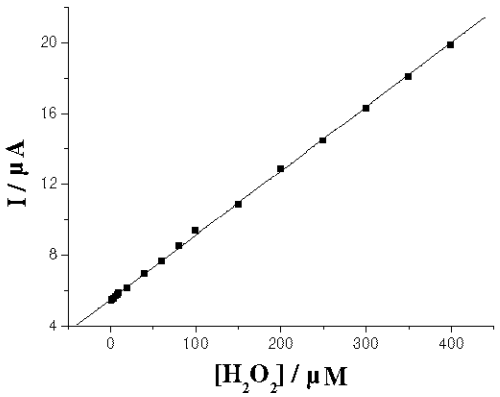

Fig. 5: The linear fitting program of the reduction peak current with H_2O_2 concentration from 1.0 × 10^{-7}~4.0×10^{-4} M.

Measurements: Electrochemical experiments were carried out with a Potentiostat / Galvanostat 283 (Princetin Applied Research, USA) and a three-electrode system. The working electrode was the modified PG disk electrode. A Saturated Calomel Electrode (SCE) was used as the reference electrode and all potentials reported here were referred to it. A platinum wire electrode served as the counter electrode. The buffer solutions were purged with purified nitrogen for at least 10 min and then a nitrogen blanket was maintained during experiments.

RESULTS AND DISCUSSION

Figure 1a shows the cyclic voltammogram of the Mb-PHB modified PG electrode in a pH 5.0 acetate buffer solution at scan rate of 200 mV s^{-1}. A pair of redox peaks is observed at -222 and -300 mV, respectively. This pair of peaks is attributed to the direct electron transfer between the heme group of Mb and electrode[11]. In contrast, no peak is found with PHB alone modified PG electrode in the same condition (Fig. 1b). Cyclic voltammograms of Mb-PHB film give nearly symmetric anodic and cathodic peaks at different scan rates (Fig. 2A) and the peak current Ip is linearly varied with scan rate in the range from 20 to 1000 mV s^{-1} (Fig. 2B), which corresponds to the characteristic of a thin layer electrochemical behavior[12]. An increase in the buffer pH causes a negative shift in potentials for the formal potential $E^{o'}$ of Mb. The slope of the $E^{o'}$ versus pH is -49.4 mV pH^{-1} (Fig. 3), which is consistent with the transfer of one proton and one electron per heme group[13,14].

Cyclic voltammograms of the Mb-PHB film modified electrode before and after the addition of aliquots of concentrated H_2O_2 solution in the buffer solution (pH 5.0) are shown in Fig. 4a and b. It can be observed that in the presence of H_2O_2, the cathodic peak increases dramatically and the anodic peak almost disappears (Fig. 4b), which indicates a typical electrocatalytic reduction process of H_2O_2. The cathodic peak current increases with the increasing concentration of H_2O_2. On the contrary, reduction of H_2O_2 cannot be observed at either bare PG or PHB alone modified PG electrodes. So, the catalytic reduction of H_2O_2 is attributed to the help of Mb.

Figure 5 shows the linear relationship of the catalytic peak current with the concentration of H_2O_2 in the range of 1.0×10^{-7} to 4.0×10^{-4} M. The regression equation is y = 5.51154 + 0.03623 x, with a correlation coefficient of 0.9997. The detection limit of this

biosensor is 3.3×10^{-8} M, which is lower than that of H_2O_2 biosensor made of hemoglobin and PHB[11].

The effect of compounds that may interfere with the response of H_2O_2 has been examined. Experimental results reveal that ascorbic acid, dopamine, catechol, uric acid and epinephrine, at a concentration of 0.5 mM, do not interfere with the determination of 100 M H_2O_2.

The reproducibility of this biosensor has been evaluated and the relative standard deviation (R.S.D.) is obtained as 3.5% for 6 determinations of 100 M H_2O_2 solution. No apparent decrease in the response of H_2O_2 has been found after the modified electrode has been stored in a refrigerator at 4°C for 7 days, so this H_2O_2 biosensor has good stability. However, because PHB can be degraded by the bacteria, this kind of biosensor should be stored in sterile conditions.

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