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Direct electrochemistry and electrocatalysis of hemoglobin in poly-3-hydroxybutyrate membrane

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Abstract

Hemoglobin (Hb) can take direct electron-transfer reactions after being entrapped in poly-3-hydroxybutyrate (PHB) film. A pair of well-defined, quasi-reversible cyclic voltammetric peaks is thus obtained at an Hb–PHB modified pyrolytic graphite electrode. The anodic and cathodic peaks are located at −224 and −284 mV for a pH 5.0 acetate buffer solution. Meanwhile, the peroxidase activity of the protein in the membrane has been greatly enhanced, with the apparent Michaelis-Menten constant calculated to be 1076 μM. According to the direct electron transfer property and enhanced peroxidase activity of Hb in the membrane, a Hb–PHB based hydrogen peroxide biosensor is prepared, with a linear range 6.0×10^{-7} to 8.0×10^{-4} M. The pathway of reductive dehalogenation of trichloroacetic acid is also discussed in detail. The highly reduced form of Hb produced in PHB film can be used to dechlorinate di- and monochloroacetic acid. The catalytic ability of Hb toward the reduction of nitric oxide has been investigated as well. Due to its biodegradability, low cost, chemical inertness, and especially its biocompatibility and non-toxicity, PHB would be a desirable film in the sensor field.

Keywords: Hemoglobin; Poly-3-hydroxybutyrate; Direct electrochemistry; Electrocatalysis

1. Introduction

Direct electrochemistry of proteins or enzymes can provide a good model for mechanistic studies of their electron transfer activity in biological systems. Protein film voltammetry (PFV) (Armstrong et al., 1997) affords a relatively new approach to study the details of electron transfer and the coupled reactions in proteins. Recently, great progress in this field has proved that films modified on electrodes may provide a favorable microenvironment for the proteins to directly exchange electrons with underlying electrodes, and thus afford a new opportunity for the detailed study of the enzyme electrochemistry (Armstrong et al., 1997; Sucheta et al., 1992; Rusling, 1998). Successful approaches have included cast films of proteins with insoluble surfactants (Rusling and Nassar, 1993; Yang and Hu, 1999; Fan et al., 2000b), biological organic substances (Fan et al., 2002; Liu et al., 2004; Shang et al., 2003b), inorganic membranes (Fan et al., 2000c, 2001a,b,c), and films of proteins and polyions grown layer-by-layer (Lvov et al., 1998; Ma et al., 2000; Shang et al., 2003a). Also, protein-containing or enzyme-containing thin films modified on electrode surface have potential applicability in fabricating biosensors, biomedical devices, and enzymatic bioreactors (Turner et al., 1987; Chaplin and Bucke, 1990). Achieving direct electron exchange between redox proteins or enzymes and electrodes simplifies these devices by removing the requirement of chemical mediators, and thus has a great significance in preparing the third generation biosensors (Gorton et al., 1999).

Poly-3-hydroxybutyrate (PHB), a linear polymer of beta-hydroxylate, is produced within bacterial cytoplasm as energy reserve by a range of prokaryotic cells (Beun et al., 2002; Majone et al., 1999; Van Loosdrecht et al., 1997). Due to its good property of biodegradability (Manna and

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Paul, 2000; Murase et al., 2001; Majid et al., 2002; Wang et al., 2002; Reddy et al., 2003; Bonartseva et al., 2003), PHB has been widely used as degradable plastics, and has an extensively application in medicine, membrane technology, and other biotechnology (Van Loosdrecht et al., 1997; Reddy et al., 2003). Besides biodegradability, the distinct advantages of PHB, such as its low cost, chemical inertness, and especially its biocompatibility and non-toxicity, also made it a significant material for the immobilization of biomolecules and even cells. For instance, PHB has been used as a good matrix to regenerate the rat sciatic nerve cells (Hazari et al., 1999). Thus, PHB might be a suitable material for this study.

In this paper, hemoglobin (Hb) is incorporated in PHB membrane and is further modified on pyrolytic graphite electrodes. Hb–PHB films can show direct, reversible electrochemistry for heme Fe^{III}/Fe^{II} redox couples. The electrochemical catalytic reductions of hydrogen peroxide (H_2O_2), and trichloroacetic acid (TCA) have been observed, showing the potential applicability of the films as biosensor. Furthermore, the mechanism of the reduction of TCA is discussed in detail. The catalytic activity of the protein towards nitric oxide (NO) has also been studied.

2. Experimental

2.1. Chemicals

Human hemoglobin (M_W = 66,000) and poly-3-hydroxybutyrate (M_W = 3155) were obtained from Sigma. They were all used without further purification. Other chemicals were all of analytical grade. The buffer solutions with different pH values were prepared as follows: pH 3.0 Gly–HCl solution; pH 4.0–5.0 NaAc–HAc solution; pH 6.0–8.0 NaH_2PO_4–Na_2HPO_4 solution; pH 9.0–10.0 Gly–NaOH solution. 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 MΩ cm^{-1} and stored in the refrigerator at the temperature of 4 °C.

2.2. Preparation of Hb–PHB film

Pyrolytic graphite (PG) electrode was prepared by putting a PG rod into a glass tube with fixing it by epoxy resin. Electrical contact was made by adhering a copper wire to the rod with the help of Wood alloy.

Prior to coating, the PG electrode was firstly polished on rough and fine sand papers. Then its surface was polished to mirror smoothness 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. Right before preparing the films, the dispersion was ultrasonicated for another 10 min.

To obtain the best cyclic voltammogram (CV) of protein–PHB films, the experimental conditions for film casting, such as the concentration of Hb, the ratio of Hb/PHB, and the total volume of Hb–PHB dispersion, were optimized. Typically, 10 μL of the dispersion containing 1.2×10^{-5} M Hb and 0.5 mg mL^{-1} PHB was spread evenly onto PG electrodes for preparing Hb–PHB films. A small bottle was fit tightly over the electrode so that water evaporated slowly and more uniform films were formed. Films were then dried overnight in air.

2.3. Apparatus and procedures

Electrochemical experiments were carried out with a Potentiostat/Galvanostat 283 (Princetin Applied Research, USA) and a three-electrode system. A one-compartment glass cell with a modified PG working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode were used for the measurements, with a working volume of 5 mL. All the following potentials reported in this work are versus SCE. Buffer solutions were purged with purified nitrogen, and a nitrogen blanket maintained during scans.

UV–vis absorption spectroscopy was performed on a Model UV-2201 spectrophotometer (Shimidz, Japan). The UV–vis absorption spectra measurements were performed in a 0.2 mg mL^{-1} Hb or mixed solution of 0.2 mg mL^{-1} Hb and 0.1 mg mL^{-1} PHB solution (Hb maintained 0.2 mg mL^{-1} in the test samples).

3. Results and discussion

3.1. Electrochemical behaviors

PHB might provide a desirable membrane environment for Hb to undergo facile electron-transfer reactions. The electrochemical reactions of entrapped Hb have been examined by using cyclic voltammetry (CV) method. Experimental results reveal that Hb can give a pair of well-defined, reversible CV peaks with the formal potential at about −0.25 V versus SCE (Fig. 1b). The peak potential separation is only 60 mV at the scan rate of 200 mV s^{-1}, which indicates a fast heterogeneous electron transfer process. And the peaks are located at the potentials characteristic of the heme Fe^{III}/Fe^{II} redox couples (Rusling and Nassar, 1993; Huang et al., 1996; Ferri et al., 1998). In contrast, no voltammetric peak can be observed for the bare PG electrode or PHB-alone modified PG electrode in the same potential window (Fig. 1a). Thus, it can be reasonably concluded that the redox reactions at the Hb–PHB modified PG electrodes are contributed from the electroactive couples in heme protein.

The CV reduction and oxidation peaks currents for immobilized Hb are found to increase linearly with potential scan