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A hydrogen peroxide biosensor based on the direct electron transfer of hemoglobin encapsulated in liquid-crystalline cubic phase on electrode

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ABSTRACT

Liquid crystal cubic phase formed with monoolein has been used as immobilizing matrix to host redox protein hemoglobin on glassy carbon electrode surface. The promoted direct electron transfer between hemoglobin and electrode was observed and a large average kinetic electron transfer rate constant k_s of 3.03(±0.02) s^{-1} was estimated. The electrode modified with cubic phase containing hemoglobin retains the bioactivity of hemoglobin and shows excellent bioelectrocatalytic activity to the reduction of hydrogen peroxide with a small apparent Michaelis–Menten constant of 0.25(±0.03) mM. A novel reagentless hydrogen peroxide biosensor was constructed using the hemoglobin-containing cubic phase modified electrode and the proposed hydrogen peroxide biosensor shows a linear range of 7.0–239 μM with a detection limit of 3.1(±0.2) μM and good stability and reproducibility.

1. Introduction

Since the very first two reports on direct electron transfer (DET) between cytochrome *c* and gold and tin doped indium oxides electrodes in 1977 by Hill [1] and Kuwana [2], respectively, DET reactions between native redox-active proteins/enzymes and electrodes have attracted considerable attention in the past several decades [3–5]. Studies on DET process of protein–electrode system not only can serve as a model system to give some information about the electron transfer mechanisms between proteins in biological and physiological systems, but also provide a platform without redox relays (mediators) for fabricating bioelectronic devices such as biosensors, bioreactors, biomotors, and biofuel cells. However, it is usually difficult to achieve the direct electron transfer between the redox proteins/enzymes and conventional bare electrodes due to some reasons including unfavorable orientation, long distance between redox center and electrode, and adsorption denaturation [6,7]. So, it is intriguing to develop suitable matrix to immobilize redox proteins/enzymes on electrode surface and, consequently, preserve the bioactivities of redox proteins/enzymes to make practical bioelectronic devices mentioned above.

A number of composite films such as nanomaterials [8–12], room temperature ionic liquids (RTIL) [13–17], surfactants [18–20], and clays [21–24] have been developed to incorporate redox proteins. Liquid-crystalline lipid cubic phase formed by polar lipids in aqueous media is one promising exception of model membranes, which is optically isotropic, highly viscous, thermodynamically stable in the laboratory for several months and easily prepared. Their internal structure consists of one congruent lipid bilayer, forming a three-dimensional and well-ordered structure interwoven by aqueous channels with a diameter about 5–6 nm. Moreover, they are stable in the presence of excess water [25–29]. From the bioelectrochemical point of view, these special characteristics make lipid cubic phase more preferable to host the biocatalysts (proteins or enzymes) on the electrode surface for electrochemical measurements in aqueous solution. For example, biocatalyst moleculars can be attached to the lipid moleculars and the high viscosity of cubic phase easily allows to fabricate the biosensing surface via casting biocatalyst-containing cubic phase mixture onto electrode surface, while the water channels will allow the enzyme substrates and products to diffuse freely through the cubic phase system. Cubic phase have already been successfully used to accommodate enzyme or synthetic catalysts on electrode surface [30–38]. Monoolein (1-monooleoyl-rac-glycerol, MO) is a typical example of

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a lipid forming such a cubic phase. It is highly viscous, transparent and at hydration over 20% is stable in contact with water [32–38].

Heme protein hemoglobin (Hb), which consists of four subunits of polypeptide enzymes, and a heme (iron porphyrin) group in each subunit acts as the active center. The electron transfer between a heme protein (e.g., myoglobin, hemoglobin) in solution and the bare electrode is either not observed or is very slow [39]. In this work, glassy carbon electrode covered by liquid-crystalline cubic phase formed by monoolein is proposed as the matrix for entrapping Hb and the DET between Hb and electrode is investigated. Based on its DET behavior and the bioelectrocatalysis activity to H_2O_2 reduction, a third-generation biosensor for H_2O_2 is established.

2. Experimental

2.1. Materials and chemicals

Monoolein (1-monooleoyl-rac-glycerol, 99%) was obtained from Sigma Chemical Co., hemoglobin (Hb) (Bovine blood, MW = 67,000) was purchased from J&K Chemical Ltd. and used as received. Hydrogen peroxide (H_2O_2, 30% w/w) solution was obtained from Shanghai Chemical Reagent Company (Shanghai, China). The dilute H_2O_2 solution was prepared daily. A 0.1 M phosphate buffer solution (pH 7.0) was used as supporting electrolyte and prepared by mixing the stock solutions of 0.1 M Na_2HPO_4 and KH_2PO_4. All other chemicals were of analytical grade and used without further purification. All solutions were prepared with double-distilled water.

2.2. Apparatus

All the electrochemical experiments were carried out on CHI660C potentiostat (CHI, Shanghai) with a conventional three-electrode cell. A glass carbon electrode or modified electrode was used as the working electrode, an Ag/AgCl as the reference electrode, and a platinum wire as the counter electrode. The experimental solutions were bubbled with highly pure nitrogen for 30 min to deoxygenate and kept under nitrogen atmosphere during the electrochemistry measurements. The electrochemical measurements were performed at room temperature and repeated minimum three times. Small angle X-ray diffraction (XRD) experiments were performed using a Shimadzu XRD-6000 X-ray diffractometer equipped with Cu Kα radiation ($\lambda = 0.154060$ nm) with a scanning rate of $0.02^\circ\ s^{-1}$ and 2θ ranges from 0.2° to 5°. A pH3-C pH meter (Shanghai, China) was used to adjust the desired pH value.

2.3. Preparation of monoolein cubic phase

The cubic phase was prepared by mixing monoolein (about 10 mg) and an appropriate amount of pure water or hemoglobin solution (2 mg/μL)in a centrifuge tube. The centrifuge tube was tightly sealed, followed by centrifugation for about 1 h at 4500 *g* for the contents to equilibrate. The ratio of the components was chosen according to the phase diagram for the monoolein–water system and it corresponds to a diamond type of cubic phase [25,32,40–42]. After centrifugation, a transparent, highly viscous and optically isotropic cubic phase was obtained. The cubic phase displays sanguine due to the color of hemoglobin.

2.4. Preparation of the Hb-cubic phase modified GC electrode

Prior to use, glassy carbon electrode (GC, ∅ = 3 mm) was polished on a polishing cloth with 0.3 and 0.05 μm alumina slurry, respectively. The electrode was carefully rinsed with double-distilled water and sonicated in ethanol and water for 2 min, respectively, and then let it dry at room temperature. The cubic phase was spread on the electrode surface using a spatula and the layer thickness was adjusted to 0.3 mm. In order to determine the exact amount of cubic phase on the electrode, the GC electrode was weighed before and after casting, and the amount was 5 mg. The electrode modified with cubic phase was immersed in the PBS which was deoxygenated and was kept in this solution for 20 min before each experiment to equilibrate the gas concentration between the cubic phase and solution.

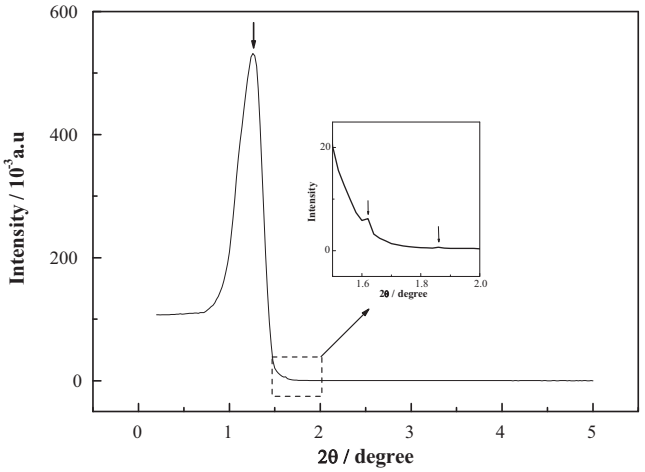

Fig. 1. Small-angle X-ray diffraction patterns of the MO–Hb–H_2O cubic phase system.

3. Result and discussion

3.1. Characterization of MO cubic phase

The phase behavior of MO cubic phase is well documented in literatures. MO forms reverse-type (i.e., water in oil) cubic phase structures in water. The formed phase are reversed isotropic and two types of liquid crystals (i.e., lamellar and cubic) are present. Two parts, corresponding to the biocontinuous structures of *Ia3d* and *Pn3m* symmetry, exist in the cubic phase domain of MO. Among these two structures, *Pn3m* structure exists only at high concentration of water (at high hydration level) and thus is stable in excess water, which is an important advantage for elaborating sensors of molecules present in water. At 20 °C, 44% water can be incorporated into the monoolein cubic phase, which allows an easy introduction of water-soluble moleculars such as protein/enzyme into liquid crystal matrix [25,30,32,40,41].

Hemoglobin-doped MO cubic phase was characterized with small-angle X-ray diffraction. One very strong diffraction peak at 2θ angle of 1.26° and two very weak peaks located at 1.64° and 1.81° are recorded in Fig. 1. According to Bragg equation ($2d \sin\theta = n\ \lambda$, $n = 1$), the Bragg reflections of the MO–Hb–H_2O system were indexed as (1 1 0), (2 0 0) and (2 1 1), and the corresponding Miller indices were 70.1, 53.8, and 49.1 Å, respectively. These X-ray diffraction data indicates that the cubic phase was the primitive cubic lattice of space group *Pn3m* [42–45], and the introduction of Hb does not change either the structure of the matrix or the lattice parameters values.

3.2. Direct electrochemistry of Hb immobilized in the lipid cubic phase

The cyclic voltammograms (CVs) of bare GC (a), MO/GC (b) and Hb–MO/GC (c) electrodes recorded in deoxygenated 0.1 M PBS (pH