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Liquid phase deposition of hemoglobin/SDS/TiO_2 hybrid film preserving photoelectrochemical activity

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ABSTRACT

This work demonstrates that liquid phase deposition (LPD) technique provides a novel approach to the immobilization of hemoglobin (Hb) in TiO_2 film for studying the direct electron transfer of Hb. Using the LPD process, a hybrid film composed of Hb, TiO_2 and sodium dodecylsulfonate (SDS) is successfully prepared on the electrode surface. The surface morphology of as-deposited Hb/SDS/TiO_2 film shows a flower-like structure. The cyclic voltammetric measurement indicates that the LPD hybrid film facilitates the electron transfer of Hb, which yields a pair of redox peaks prior to the characteristic voltammetric peaks of TiO_2. Due to the electrocatalytic activity of Hb towards H_2O_2, the Hb/SDS/TiO_2 hybrid LPD film can be utilized as an H_2O_2 sensor, showing a sensitive response linearly proportional to the concentration of H_2O_2 in the range of 5.0×10^{-7}–4.0×10^{-5} mol/L. At the same time, the Hb/SDS/TiO_2 hybrid film preserves the photoelectrochemical activity of TiO_2. The photovoltaic effect on the electrochemical behavior of Hb/SDS/TiO_2 film is observed after long-time UV irradiation on the film, which could improve the calibration sensitivity for H_2O_2.

1. Introduction

Due to the deep burying of electroactive centers of proteins in the molecule, the direct electrochemistry of hemoglobin (Hb) is well-known to be sluggish on conventional electrodes. To facilitate the electron transfer between protein and electrode, numerous efforts have been devoted to provide a desirable protein–electrode interface environment in which the electron transfer can be probed [1]. Although the direct electron transfer of Hb has been achieved on various film-coated electrodes or chemically modified-electrodes such as sol–gel films [2,3], ion liquids [4], clay [5], nanomaterials [6–9], and self-assembled monolayers [10–12], there are two routine methods namely cast and adsorption used to immobilize Hb on the electrode surface.

As one of the most important semiconducting electrode materials, TiO_2 film has been demonstrated as an attractive material for hemoglobin immobilization [13]. It has been proven that TiO_2 film could not only offer a friendly platform to assemble protein molecules, but also enhance the electron transfer between protein and electrode [14]. Interestingly, the particle size and porosity in TiO_2 phytate films have been demonstrated to have a considerable effect on the adsorption and transport of proteins in the film by voltammetric methods [15]. Meanwhile, Hb has been entrapped in a TiO_2 sol–gel matrix or adsorbed on nanocrystalline TiO_2 films to construct amperometric H_2O_2 biosensors [16], NO electrochemical biosensors [17], or amperometric antioxidant sensors [18]. Various types of TiO_2 materials such as highly ordered mesoporous TiO_2 [19], TiO_2 nanotubes [20,21], TiO_2 nanorods [22], and TiO_2 whisker [23] have been prepared for hemoglobin immobilization and utilized for the study of direct electron transfer of Hb. On the other hand, TiO_2 is a well-known semiconducting photocatalyst absorbing UV light. It has been discovered that the electrocatalytic activity of UV-irradiated Hb in TiO_2 films could be enhanced due to the photovoltaic effect of TiO_2 [23–25].

Liquid phase deposition (LPD) provides a flexible wet-chemical approach to the deposition of metal oxide films [26], which is particularly useful for preparing TiO_2 film-coated electrodes on various conducting substrates for voltammetric measurements [27,28]. The as-prepared LPD TiO_2 films exhibit high photoelectrochemical activity and can be applied to the degradation of organic pollutants [29,30]. Meanwhile, LPD technique also provides a ready process for forming TiO_2-based hybrid thin films by simply doping organic or biological materials into the deposition solution [31–35]. Recently, we prepared SDS/TiO_2 hybrid film and observed that SDS could improve the electrocatalysis of Hb towards H_2O_2 after Hb was adsorbed on the hybrid film [36], although we did not observe the direct electrochemistry of adsorbed protein.

In this work, we prepared a hybrid film of TiO_2, SDS and Hb via the LPD process, which was demonstrated as a novel strategy to the immobilization of Hb on the electrode surface. In as-prepared hybrid film, TiO_2 exhibited two interesting impacts on the electrochemical behavior of Hb. Firstly, TiO_2 facilitated the electron transfer between Hb and electrode. Using such a LPD Hb/SDS/TiO_2 film, the direct electrochemistry of Hb was successfully investigated and an Hb-based

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electrochemical biosensor for H_2O_2 was developed. Secondly, the photoelectrochemical activity of TiO_2 in the hybrid film showed a photovoltaic effect on the electrochemical behavior of Hb. The bioelectrocatalysis of protein in Hb/SDS/TiO_2 film towards H_2O_2 was improved under UV irradiation, which was useful to develop more sensitive H_2O_2 biosensors.

2. Experimental

2.1. Reagents

Bovine Hb was purchased from Sigma Chemical Reagent Co., USA. Sodium dodecylsulfonate (SDS) and boric acid were from Sinopharm Chemical Reagent Co. Ltd, China. Ammonium hexafluorotitanate was obtained from Shanghai Sanaisi Reagent Co., China. Hydrogen peroxide was obtained from Shanghai Experimental Reagent Co., China. Phosphate buffer solution (PBS) was prepared by mixing the stock solutions of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. All solutions were prepared with doubly distilled water.

2.2. Deposition of Hb/SDS/TiO_2 film

The Hb/SDS/TiO_2 film was deposited on a glassy carbon (GC) electrode surface via the LPD method. Prior to film deposition, the GC electrode surface was polished to a mirror-like smoothness with sand papers, and then washed by ultrasonication in ethanol and distilled water for 5 min, respectively. After drying with nitrogen gas, the GC electrode was immersed vertically in a polytetrafluoroethylene beaker containing a deposition solution of 0.1 M $(NH_4)_2TiF_6$, 0.2 M H_3BO_3, 60 mM SDS and 2 mg/mL hemoglobin at 50 °C for 10 h. Finally, the electrode was rinsed with doubly distilled water and then dried with a nitrogen gas flow. The LPD SDS/TiO_2 film was prepared using the same procedure except that Hb was absent in the deposition solution.

2.3. Apparatus and procedures

The morphology of LPD hybrid films was characterized with a Sirion 200 field-emission scanning electron microscopic (FE-SEM) instrument (FEI, the Netherlands). The UV–visible absorption spectra were measured with a TU-1900 UV–visible spectrometer (Beijing Purkinje General Instrument Company, China). The electrochemical measurements were carried out in a conventional three-electrode cell controlled with a CHI 660A electrochemical working station (Shanghai Chenhua Instrument Co., China). A LPD film-coated GC with an exposed area of ca. 0.0706 cm^2 was employed as the working electrode. A platinum wire and a saturated calomel electrode (SCE) served as the auxiliary and reference electrodes, respectively. All the potentials were referred to SCE. All electrochemical experiments were carried out in deaerated solutions acquired by purging the solution with high purity nitrogen gas for at least 20 min.

The photoelectrochemical response of the LPD film was measured in a quartz tube containing 0.1 M Na_2SO_4 solution. The film-coated GC electrode served as the photoanode irradiated with a 15-W UV lamp with a major emission at 253.7 nm. To observe the photovoltaic effect, the Hb/SDS/TiO_2 film was irradiated with the 15-W lamp under atmosphere conditions for a period of time before being inserted in the cell for electrochemical measurements.

3. Results and discussion

3.1. Characterization of LPD hybrid films

Fig. 1 displays the FE-SEM images of LPD Hb/SDS/TiO_2 and SDS/TiO_2 films on the GC substrate surface. Different from pure LPD TiO_2 film which was composed of many spherical nanoparticles [27], both LPD Hb/SDS/TiO_2 and SDS/TiO_2 hybrid films showed a flower-like structure, attributed to the effect of doped SDS molecules [36]. Although the presence of Hb in the deposition solution did not significantly change the morphological structure of SDS/TiO_2 film, the Hb/SDS/TiO_2 film seemed to be less compact. In the present weakly acidic deposition solution, Hb was positively charged and could strongly interact with anodic SDS molecules in the SDS/TiO_2 hybrid film. As the result, a hybrid film of TiO_2, SDS and Hb was readily formed via the LPD process. On the other hand, we tried to prepare a hybrid film of Hb/TiO_2 without SDS using the same procedure. However, in the absence of SDS, Hb/TiO_2 was hardly deposited on the electrode surface via the LPD process, which might be due to the repulsion between positively charged Hb and TiO_2. We conclude that anodic SDS plays an important role in the formation of Hb hybrid film during the LPD process.

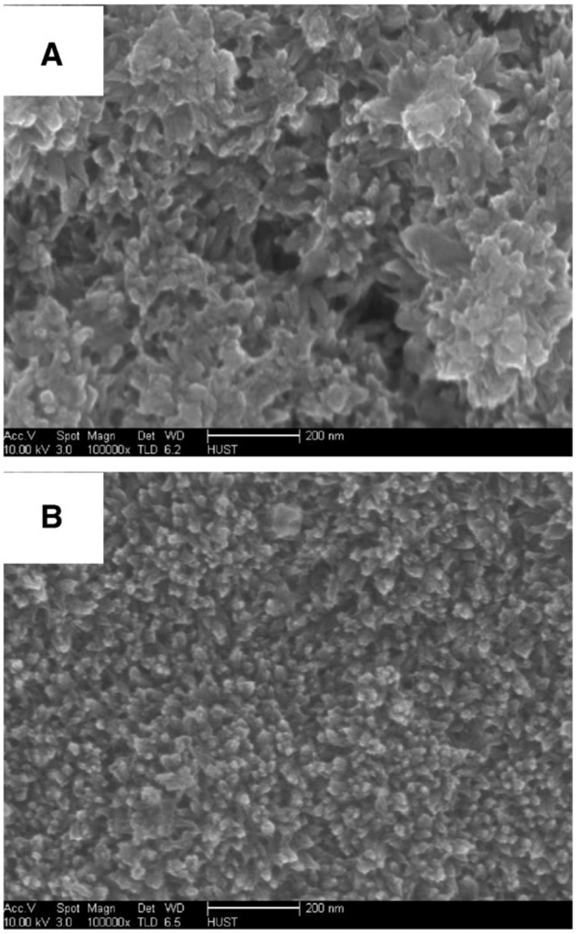

Fig. 1. FE-SEM images of LPD Hb/SDS/TiO_2 (A) and SDS/TiO_2 (B) films (bar: 200 nm).

The UV–visible spectra of LPD Hb/SDS/TiO_2 and SDS/TiO_2 films deposited on quartz were measured and compared in Fig. 2. Similar to the pure LPD TiO_2 film [35], both hybrid LPD films showed strong absorption at the wavelengths less than 350 nm. While in the presence of Hb, the absorption of hybrid film in the visible light region was obviously increased. Although this result was different from the adsorbed Hb on TiO_2 film that usually showed a characteristic absorption peak of native Hb at 406 nm [37], the increased absorption of Hb/SDS/TiO_2 in the visible light region could be assigned to the increased film thickness and reflect the interaction of Hb with SDS and TiO_2 during the deposition process for effectively immobilizing Hb in the LPD film.

3.2. Direct electron transfer of Hb in LPD hybrid film

Fig. 3 shows the cyclic voltammograms (CVs) in 0.1 M PBS (pH 7.0) recorded on the Hb/SDS/TiO_2 and SDS/TiO_2 film-coated electrodes. As