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Direct electrochemistry and electrocatalysis of hemoglobin immobilized on an interlaced $Co(OH)_2$ nanosheet-based three-dimensional macroporous film

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ABSTRACT

Hemoglobin (Hb) has been successfully immobilized on a glassy carbon (GC) electrode modified by an interlaced $Co(OH)_2$ nanosheet-based three-dimensional (3D) macroporous film prepared by a cathodic electrodeposition method. The surface morphology of the $Co(OH)_2$ film was examined by scanning electron microscopy. UV–vis spectra revealed that the Hb immobilized on the $Co(OH)_2$ film retained its native structure. A fast direct electron transfer was achieved between Hb and the underlying electrode, with an average electron transfer rate of 7.8 s^{-1}. The resulting biosensor exhibited good performance for the detection of H_2O_2, with a wide linear range from 0.4 to 200 μM, low detection limit of 0.2 μM, high sensitivity of 744 μA mM^{-1} cm^{-2}, excellent stability and reproducibility. The cathodic electrodeposition method provides a simple and efficient way to prepare a 3D nanostructured film for immobilizing protein or enzyme, and the resulting film has potential applications in biosensors, catalytic bioreactors, and biomedical devices.

1. Introduction

Studies of the direct electron transfer of redox proteins and enzymes can not only help in understanding metabolic processes in biological systems, but also establish a foundation for fabricating the third generation of electrochemical biosensors, catalytic bioreactors, and biomedical devices [1,2]. However, it is difficult for biomolecules to directly exchange electrons with an electrode surface due to the deeply buried redox-active center in the biomolecule and the generally unfavorable orientation of a biomolecule on the electrode surface [3,4]. Therefore, one of the main challenges in this field is to find a suitable immobilization matrix which can enhance the direct electron transfer between the biomolecule and the electrode.

Nanostructured metal oxides (hydrates) have attracted considerable attention due to their optical, electrical, and magnetic properties [5–7], and also have been employed in the bioanalytical area because of their high surface area, strong adsorption ability, good biocompatibility, ease of fabrication and good stability. A wide variety of nanostructured metal oxides (hydrates), such as zero-dimensional (0D) nanoparticles (NiO [8], CoO_x [9], Fe_3O_4 [10], SiO_2 [11]), one-dimensional (1D) nanowires (CuO [12]) and nanotubes (ZnO [13], TiO_2 [14]), two-dimensional (2D) nanosheets (Ni–Al layered hydroxides [15]), and three-dimensional (3D) nanostructured microspheres (ZnO [16], TiO_2 [17]) and macroporous films (TiO_2 [18,19]) have been extensively studied for use in biosensors. It has been shown that the morphology and structure of the immobilization matrix have a significant influence on the performance of the biosensor.

3D macroporous film-based biosensors exhibit excellent performance due to their high surface area for enzyme immobilization, as well as their macroporous structure which facilitates reactant transportation [18–20]. However, the reported synthesis of 3D macroporous films often rely on a template approach which is relatively complicated, in that it needs both template self-assembly and subsequent template removal steps.

In this work, we report a one-step preparation of an interlaced $Co(OH)_2$ nanosheet-based 3D macroporous film on a glassy carbon (GC) electrode surface using a cathodic electrodeposition technique, which is simple and rapid to carry out, easily controlled and environmentally friendly. Such a film is particularly promising for applications in biosensors for several reasons. Firstly, by constructing the $Co(OH)_2$ film from interlaced nanosheets standing on the electrode surface, a large effective surface area is available for biomolecule immobilization. In addition, the interlaced structure of the $Co(OH)_2$ film is stable and resistant to collapse, which inhibits the aggregation of nanomaterials on the electrode surface. Furthermore, the open, interconnected pore structure can ensure the accessibility of the substrate to biomolecules immobilized on the $Co(OH)_2$ film, increasing both the mass transport of the reactant and the mass-normalized activity [18]. However, to the best of our knowledge, whilst structured $Co(OH)_2$ films of this type have been

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employed in electrochemical capacitors [21–23] there have been no reports of their use in biotechnology applications.

In this paper, hemoglobin (Hb) was selected as a model redox protein for construction of a H_2O_2 biosensor. The direct electrochemistry and biosensor performance of Hb immobilized on the as-prepared interlaced $Co(OH)_2$ nanosheet ($Co(OH)_2$NS)-based 3D macroporous film was studied and compared with those of Hb immobilized on a $Co(OH)_2$ nanoparticle ($Co(OH)_2$NP) film, in order to highlight the advantages of the interlaced $Co(OH)_2$ nanosheet-based 3D macroporous film as an immobilization matrix.

2. Experimental

2.1. Reagents and apparatus

Bovine blood hemoglobin (Hb, M_W 64,500) from Sigma was used without further purification. Phosphate buffer solutions (PBS) (0.1 M) of various pH values were prepared by mixing stock solutions of Na_2HPO_4 and NaH_2PO_4. All other chemicals were of analytical grade and used without further purification. Doubly distilled water was used to prepare all solutions and to rinse the electrodes.

Electrochemical measurements were performed using a CHI 660B electrochemical workstation (Chenhua, China). A conventional three-electrode system was used with the modified electrode as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (3 M KCl) electrode as the reference electrode. High-purity nitrogen was bubbled through all solutions for at least 20 min to ensure thoroughly anaerobic conditions, and a nitrogen atmosphere was maintained over the solutions during measurements. UV–vis spectra were recorded using a UV-2501PC spectrophotometer (Shimadzu, Japan) on an indium tin oxide (ITO) coated glass electrode. Field-emission scanning electron microscopy (FESEM) images were obtained using a S-4700 field emission scanning electron microscope (Hitachi, Japan). All measurements were performed at room temperature (approximately 20 °C).

2.2. Electrodeposition

The electrodeposition was performed in a standard three-electrode glass cell. The working electrode was a GC electrode with a diameter of 3 mm. A platinum wire was used as the counter electrode and an Ag/AgCl (3 M KCl) electrode as the reference electrode. Prior to electrodeposition, the GC electrode was successively polished on a polishing cloth with 1.0, 0.3 and 0.05 μm alumina powder and rinsed with deionized water. The electrode was then dried with a purified nitrogen stream. The $Co(OH)_2$NS and $Co(OH)_2$NP films were electrodeposited from 0.1 M $Co(NO_3)_2$ solution at a current density of 15 mA cm^{-2} using different electrodeposition times (see Section 3.1), using a Chenhua CHI 660B electrochemical workstation. The electrodeposition process of the $Co(OH)_2$ film includes an electrochemical reaction and a precipitation reaction as follows [21]:

$$NO_3^- + 7H_2O + 8e^- \rightarrow NH_4^+ + 10OH^-, \quad (1)$$

$$Co^{2+} + 2OH^- \rightarrow Co(OH)_2 \quad (2)$$

2.3. Hb immobilization

To prepare the Hb/$Co(OH)_2$NS/GC electrode, the $Co(OH)_2$NS/GC electrode was immersed in 0.1 M pH 7.0 PBS containing 3.0 mg mL^{-1} Hb at 4 °C for 6 h. Then, the modified electrode was rinsed thoroughly with doubly distilled water to remove unbound Hb and stored at 4 °C in a refrigerator when not in use.

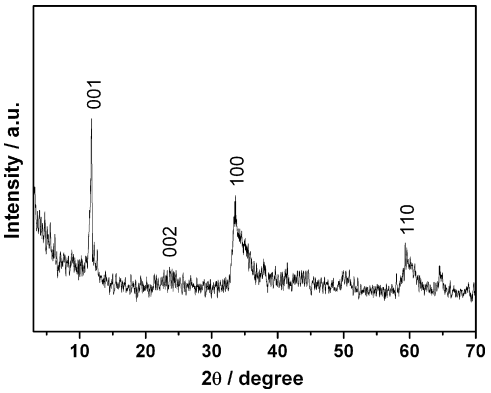

Fig. 1. XRD pattern of the electrodeposited $Co(OH)_2$NS film.

A Hb/$Co(OH)_2$NP/GC electrode was also prepared using a similar procedure.

3. Results and discussion

3.1. Characterization of $Co(OH)_2$ film

Fig. 1 shows the XRD pattern of the electrodeposited $Co(OH)_2$ film. The peaks at 2θ values of 11.4° (0.775 nm), 22.7° (0.381 nm), 34.0° (0.268 nm) and 59.6° (0.155 nm) can be respectively assigned to the (0 0 1), (0 0 2), (1 0 0) and (1 1 0) reflections of an α-$Co(OH)_2$ phase, which has a layered structure [23]. In order to investigate the formation and growth of the $Co(OH)_2$ film, FESEM images of the $Co(OH)_2$ films formed after different electrodeposition times on the GC electrode surface were obtained as shown in Fig. 2. The electrodeposition reaction time has a significant influence on the morphology of the $Co(OH)_2$ film. Nanoparticles ($Co(OH)_2$NP) were obtained after electrodeposition for 5 s (Fig. 2A), whereas erect interlaced nanosheets ($Co(OH)_2$NS) formed on the GC electrode surface after reaction for 20 s (Fig. 2B). The inset of Fig. 2B is a higher magnification image of the $Co(OH)_2$ film, showing its interlaced nanosheet-like characteristics, with an open and interconnected macropore structure, with pore diameter of hundreds of nanometers. The nanosheets continued to grow if the electrodeposition time was further extended, forming larger pores after electrodeposition reaction for 30 s (Fig. 2C). After immobilization of Hb on the $Co(OH)_2$NS film, the FESEM image of the resulting film (Fig. 2D) shows that it retained the nanosheet-based 3D macroporous structure of the precursor film (Fig. 2B). However its surface was much rougher than that of the precursor, consistent with immobilization of Hb in a Hb/$Co(OH)_2$NS film.

Electrochemical impedance spectra (EIS) were employed to monitor the growth of the $Co(OH)_2$ film using $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ at its formal potential as a redox probe. Fig. 3 shows the EIS in the form of Nyquist diagrams at the $Co(OH)_2$ film electrodes fabricated with different electrodeposition reaction times. The electron-transfer resistance (R_{ct}), which controls the electron-transfer kinetics of the redox probe at the electrode interface [24], can be calculated according to the equivalent circuit (inset in Fig. 3). At the $Co(OH)_2$ film electrodeposited for 5 s, the redox process of the probe showed a R_{ct} value of about 88 Ω (curve a). With increasing electrodeposition times of 20 s and 30 s, the R_{ct} values of the resulting $Co(OH)_2$ films increased to 148 Ω (curve b) and 195 Ω (curve c) respectively. The latter R_{ct} value is unsuitable for the electron transfer between an enzyme and an electrode and therefore we chose the $Co(OH)_2$NS film electrodeposited for 20 s as the matrix for all enzyme immobilization studies.