Electrochemistry and Electrocatalysis with Hemoglobin in Hollow Polyelectrolyte Fibrous Mats

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ABSTRACT: To form the PS/PAH fibers, positively charged poly(allylamine hydrochloride) (PAH) was coated on the surface of polystyrene (PS) fibers. Then, the ordered Hb-PS/PAH film was prepared by the self-assembly of hemoglobin (Hb) on the surface of fibers and characterized by electrochemical and spectroscopic methods. Cyclic voltammetry of the self-assembled Hb-PS/PAH films modified on glassy carbon electrode (GCE) displayed a quasi-reversible electrochemical response in pH 5.0 buffers. Moreover, the Hb-PS/PAH films also exhibited the electrocatalytic activity to the reduction of H_2O_2. Consequently, the Hb-PS/PAH films are favorable for the direct electrochemistry of heme containing proteins, suggesting their potential application as the promising sensitive biosensor. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 1613–1617, 2010

Key words: biopolymers; nanocomposites; nanotechnology; proteins; self-assembly

INTRODUCTION

Because of the very slow rates of electron transfer between hemoglobin (Hb) and solid electrodes, much effort has been made to enhance the electron transfer rate of Hb. Study on protein-containing thin film-modified electrode has a great significance in preparing biosensors, biomedical devices, and enzymatic bioreactors.[1–4] A series of materials, such as insoluble surfactants, biological organic substances, polyelectrolyte, clay surfactant composites, inorganic membranes, and polyions, have been used to fabricate ordered modification films.[5–8] When compared with the case of the proteins in solution on bare solid electrodes, most of these modified electrode films could facilitate electron transfer between metalloproteins and electrodes.

Considering the biocompatible and biodegradable, polymers, especially polymer fibers, show broad application in the sensors, controlled drug delivery and tissue growth applications.[9,10] Hu and coworkers[11] introduced electrochemical property of multilayered composite films formed by incorporating surfactant-polymer with hemoglobin. Additionally, much attention has been paid on using natural polymers as an enzyme immobilization matrix for biosensor construction.[12,13]

In the recent years, an increasing interest has been paid on the direct electrochemistry of layer-by-layer (LBL) proteins-polyelectrolyte films.[14–18] The incorporating of heme proteins with DNA, poly(styrene sulfonate) (PSS), or poly(ethyleneimine) (PEI), etc. could be readily used to modify electrodes to enhance the electrochemical property of Hb.[19]

On the basis of the earlier description, in this study, we first prepare the polyelectrolyte fibers PS/PAH. The coral template polystyrene (PS) fibers are obtained by electrospinning technique. Poly(allylamine hydrochloride) (PAH) is selected as shell material because it is favorable to form thin film. PAH layers are assembled on the PS fibrous surface to form the PS/PAH, which could be used as a building block or film-forming materials in preparing protein films. Then, Hb is adsorbed on surface of the polyelectrolyte fibers to form the Hb-PS/PAH composite, which is then casted on glassy carbon electrodes (GCE). The results indicate that the Hb-PS/PAH films may accelerate the electron transfer between Hb and electrodes. Moreover, the film can be used to promote the catalytic reduction process of H_2O_2,

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showing the potential application of the films as biosensor.

EXPERIMENTAL SECTION

Reagents

Bovine hemoglobin was purchased from Sigma. All other reagents used were analytical grade. Acetate buffer solution (pH 5.0, 0.05*M*) was prepared by mixing HAc and NaAc. All other solutions were freshly prepared by using ultrapure water, which is obtained with a Milli-Q purification system (Branstead, USA) to a specific resistance above 18 MΩ/cm.

Apparatus and procedures

Electrochemical measurements are performed on a CHI 660B electrochemical workstation at room temperature (22°C ± 2°C) under the nitrogen atomsphere. A three-electrode system is used in the relative electrochemical study, which contains the Hb-PS/PAH film-modified glassy carbon electrode (GCE) as the working electrode, a Pt electrode as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All solutions are purged with high-purified nitrogen for at least 20 min before each experiment. The nitrogen environment is then maintained over the solutions in the electrochemical cell during the respective measurements. Scanning electron micrographs (SEM) are obtained by using scanning electron microscopy (Hitachi, S-3000N) with an accelerating voltage of 20 kV. Transmission electron micrographs (TEM) were obtained by using JEM2000EX (JEOL).

Fabrication of polystyrene fibrous mats with electrospinning technique

Polystyrene fibrous mats are prepared from tetrahydrofuran (THF)/*N*,*N*-dimethylformamide (DMF) mixture solution and polystyrene polymer. As a typical procedure, 1.5 g polystyrene (M_w = 185,000) is immersed into 9.32 mL THF/DMF solution for 2 h to get a viscous gel. Then, it is quickly loaded into a syringe equipped with a five-gage stainless needle, which is connected to high-voltage up to 30 kV. The feeding rate of the precursor solution is controlled by using an automatic syringe pump. A plate used as the collector is placed 15 cm from the tip of the needle for the collection of the fibers. The solution on the tip of the needle is ejected as fibers towards the collector under a strong electric field.

Preparation of PS/PAH fibers

Poly(allylamine hydrochloride) (PAH) is selected as shell material because it is favorable to form thin film. PAH layers are assembled on the PS fibrous surface to form the PS/PAH. The PS mat is initially immersed into 1 mg/mL PAH aqueous solution (with 0.5*M* NaCl). In every step, 30 min is left for adsorption and then rinsed with abundant water. The diameter of PS/PAH fibers used in this work is in the range of 920–1320 nm with the average diameter of about 1000 nm, whereas the diameter of the PS fibers is in the range of 820–1220 nm with the average diameter of about 900 nm. These size data also demonstrate the formation of the polymer layer on the PS surface.

Preparation of Hb-PS/PAH films modified on GCE

Initially, to prepare the Hb-PS/PAH films modified on GCE, the fibers of PS/PAH (0.1 mg) are added into 10 mL double-distilled water, and the resultant mixture is ultrasonicated for about 20 min. Then, the PS/PAH (0.01 mg/mL) is mixed with the hemoglobin solution (1 mg/mL). Afterward, the Hb-PS/PAH film-modified electrodes are prepared by immersing the cleaned GCE electrodes in the above incubation solution for 24 h. At last, the relative electrodes are removed from the incubation solution and rinsed thoroughly with double-distilled water, which are then dried with nitrogen and stored at 4°C.

RESULTS AND DISCUSSION

Morphologies

Figure 1 illustrates the typical TEM images of PS/PAH fibers and Hb-PS/PAH composites. As shown in Figure 1, it appears that the hemoglobin has been adsorbed at the sidewall of PS/PAH.

Direct electrochemistry of Hb-PS/PAH film-modified GCE

On the basis of the earlier studies, we incorporate the biocompatible PS/PAH fiber with the Hb to form the Hb-PS/PAH composite to modify the GCE. We have further explored the electrochemical behavior of the Hb-PS/PAH film-modified GCE. Figure 2 shows the cyclic voltammograms of Hb-PS/PAH film-modified GCE in pH 5.0 acetate buffer solution, which indicates a quasi-reversible electrochemical response (curve c, Fig. 2). In contrast, the cyclic voltammogram of PS/PAH film-modified electrode in pH 5.0 acetate buffer solution shows no detectable signal (curve b, Fig. 2). Meanwhile, Hb indicates the poor or even no response under the same condition (curve a, Fig. 2). So the presence of PS/PAH is favorable for the

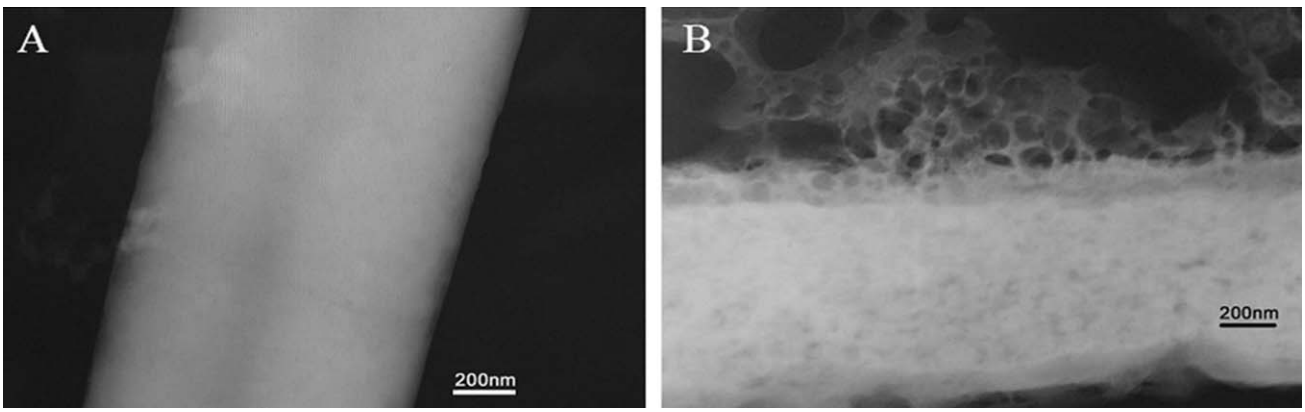

Figure 1 Typical TEM images of (A) PS/PAH and (B) Hb-PS/PAH.

proteins to exchange electrons with the modified electrode. The relative cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}) are located at −0.36 V and −0.27 V, respectively. The separation of peak potentials, ΔE_p, is 90 mV. Its formal potential (defined as the average of the anodic and cathodic peak potentials), $E^{0'}$, is −0.31 V. The value of $E^{0'}$ is close to previous report such as the incorporation of Hb within didodecyldimethylammonium bromide (DDAB)-clay film[20] and hemoglobin immobilized in a magnetic nanoparticles-chitosan film.[21]

Furthermore, we have also explored the effect of scan rates on the eletrochemical response of films. Figure 3 illustrates the cyclic voltammograms (CVs) of Hb-PS/PAH films modified GCE in pH 5.0 acetate buffer solution obtained at different scan rates and the plot of the scan rate dependent of the relative redox currents. From the results, it is noted that the reduction and oxidation peak currents for the immobilized Hb increase linearly with the scan rate between 0.05 and 0.8 V s^{-1} [Fig. 3(B)], suggesting that the reaction is surface controlled, as expected for immobilized systems.[22]

The number of electron transferred (n) in this process can be estimated according to Laviron's equation[23]:

$$I_p = \frac{n^2 F^2 A\Gamma v}{4RT} = \frac{nFQv}{4RT}$$

Γ (mol cm^{-2}) is the surface coverage of redox species on the electrode. A (cm^2) is the electrode surface area and Q (C) is the quantity of charge and calculated from the peak area of the voltammogram with the background correction. The symbols n, I_p, F, R, and T have their usual meanings. From the slope of the $I_p$versus v curve, n is calculated to be 0.96 for Hb-PS/PAH film, suggesting that the redox reaction of Hb on PS/PAH-modified GCE electrode is a single electron transfer process.

Besides, the average surface coverage (Γ) of electroactive Hb immobilized on PS/PAH films can be estimated using the equation $\Gamma = Q/nFA$. The average Γ value is calculated to be 6.0×10^{-11} mol cm^{-2} for PS/PAH films.

As we know, when $\Delta E_p <$ 200 mV, the electron transfer rate constant k_s of Hb on the modified electrode can be obtained by the following equation[24]

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\upsilon} - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT}$$

Laviron point out that the relative error of k_s is at the most about 6% if the relationship for $\alpha = 0.5$ is used. Taking a charge transfer coefficient α of 0.5, and a scan rate of 100 mV s^{-1}, the ΔE_p is 107 mV for Hb-PS/PAH film, giving an average k_s value of is 0.68 s^{-1}. The value is in the range of k_s for typical surface-controlled quasi-reversible electron transfer and is close to those reported for Hb on the other electrodes.[24]

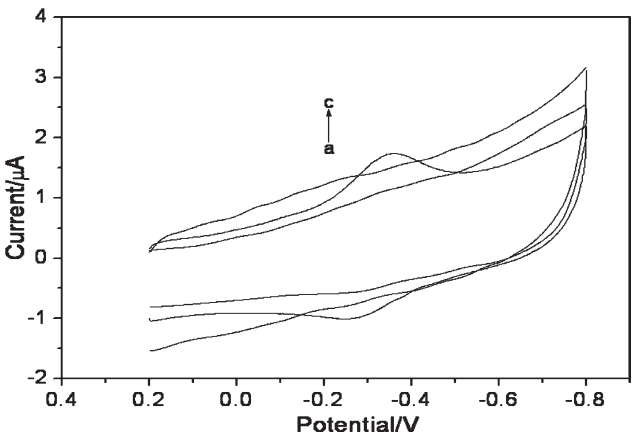

Figure 2 Cyclic voltammograms of the respective film-modified GCE in pH 5.0 acetate buffers at scan rate of 100 mV s^{-1} for (a) Hb film, (b) PS/PAH film, and (c) Hb-PS/PAH film.

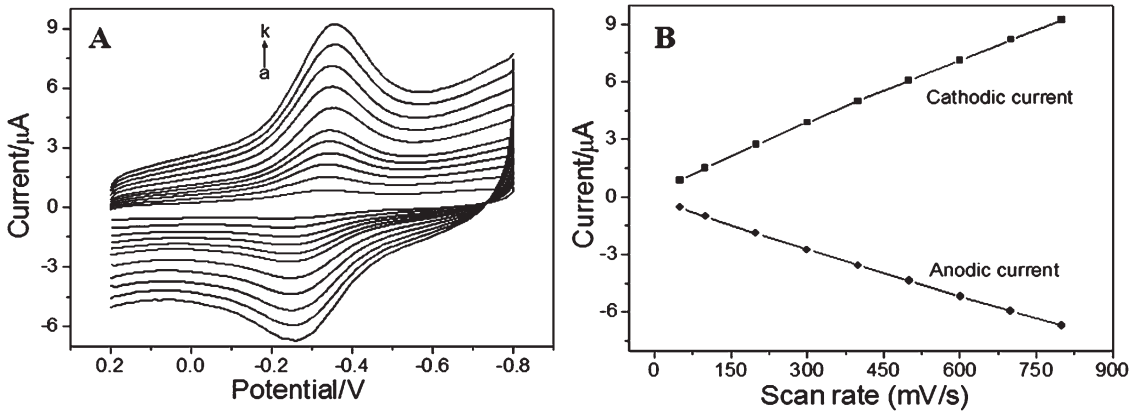

Figure 3 (A) Cyclic voltammograms of the Hb-PS/PAH film-modified GCE in pH 5.0 acetate buffers at different scan rates (from inner to outer curve): (a) 0.05, (b) 0.1, (c) 0.15, (d) 0.2, (e) 0.25, (f) 0.3, (g) 0.4, (h) 0.5, (i) 0.6, (j) 0.7, and (k) 0.8 mV s^{-1}. (B) Plot of cathodic and anodic currents vs scan rates.

It is known that the pH value influences the redox potential of Hb.[25] Figure 4 illustrates the effect of pH value of the external buffer solutions on CVs of the Hb-PS/PAH film-modified GCE. Based on the results, it illustrates that as the increasing the pH of the solution, both reduction and oxidation peak potentials show negative shift in potential for Hb-PS/PAH-modified GCE.

Furthermore, we can also observe that the formal potential $E^{0'}$ has a linear relationship with the pH values from 3.6 to 5.7, with a slope of −59.8 mV/pH for Hb-PS/PAH films. The slope value is comparable with the theoretical value of −58 mV/pH at 20°C for the single-proton transportation process, which indicates that a single protonation accompanies the electron transfer of Hb Fe(III) to the modified electrode.[26]

The GCE coated with Hb-PS/PAH films are stored in the refrigerator at 4°C for use and CVs are performed periodically. Our experimental results have revealed that these film-modified GCE electrodes are very stable. No significant decrease of the peak currents is observed after at least 2 weeks storage at 4°C.

Electrochemical catalysis

Hemoglobin is known to be capable to electrocatalyze the reduction of H_2O_2. The bioelectrocatalytic activity of the Hb-PS/PAH film-modified GCE is checked in our study. The cyclic voltammograms of the Hb-PS/PAH film-modified GCE before and after the injection of H_2O_2 solution in pH 5.0 HAc/NaAc buffers are shown in Figure 5. It can be observed that compared with the system in the absence of H_2O_2 (curve c, Fig. 5), an obvious increase of the CV reduction peak at about −0.35 V is observed when H_2O_2 is added to the relative buffer solution (curve d, Fig. 5). Besides, the gradual disappearance of the oxidation peak is also observed under the same experimental condition, indicating a typical electrocatalytic reduction process of H_2O_2. In the mean-

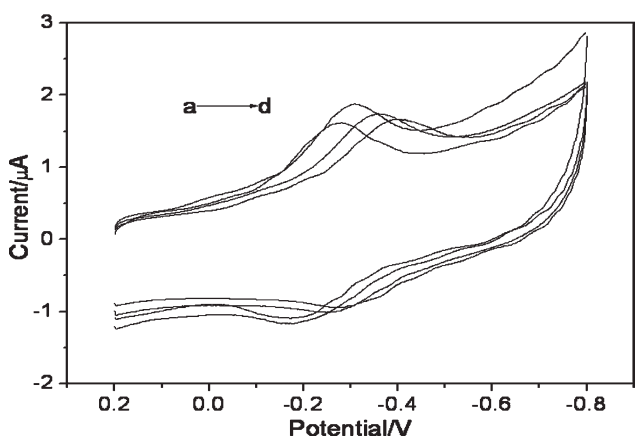

Figure 4 Influence of pH on cyclic voltammograms of the Hb-PS/PAH: (a) pH 3.6, (b) pH 4.0, (c) pH 5.0, and (d) pH 5.7.

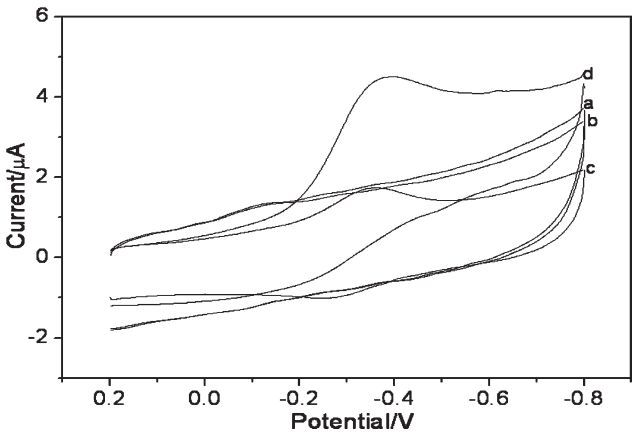

Figure 5 Cyclic voltammograms of the PS/PAH film (a,b) and Hb-PS/PAH (c,d) modified GCE electrodes in pH 5.0 acetate buffer solution at a scan rate of 100 mV s^{-1} in the absence (a,c) and presence of $1.5 \times 10^{-4} M$ H_2O_2 (b,d).

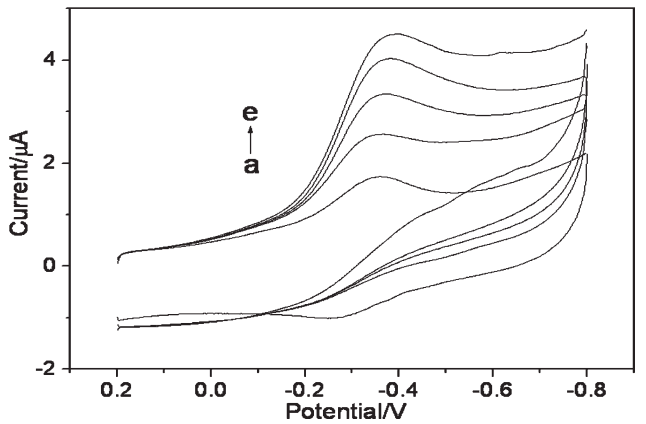

Figure 6 Cyclic voltammograms of the Hb-PS/PAH film-modified GCE electrodes in pH 5.0 acetate buffer solutions at a scan rate of 100 mV s^{-1} with the H_2O_2 concentration of 0, 4.5×10^{-5}, 8.3×10^{-5}, 1.2×10^{-4}, $1.5 \times 10^{-4}M$ (from a to e).

while, the cyclic voltammogram of PS/PAH film-modified GCE is also explored for comparison (curve a, Fig. 5). No detectable reduction current is observed after adding the H_2O_2 with the same concentration (curve b, Fig. 5).

Additionally, to illustrate the effect of various concentration of H_2O_2, we have also investigated the electrochemical responses of the Hb-PS/PAH film-modified GCE in acetate buffer solution (pH 5.0) upon various concentration of H_2O_2 (as shown Fig. 6). The results show that the reduction peak current increased as the enhancement of H_2O_2 concentration in buffer solution. The CV reduction peaks have a linear relationship with H_2O_2 concentration in the range of 3.8×10^{-6} to $1.0 \times 10^{-4}M$. At higher H_2O_2 concentration, the CV response does not increase any more. The relative detection limit (3σ)[27,28] is estimated to be $1.8 \times 10^{-7}M$, which is close to the previous report such as 0.5 μM for Hb-polymer films.[29]

CONCLUSIONS

In this study, the Hb-PS/PAH films are prepared to facilitate the direct electron transfer of Hb efficiently. Moreover, Hb-PS/PAH film exhibits an electrocatalytic activity to the reduction of H_2O_2 and can be used as an amperometric sensor for the detection of H_2O_2. The detection limit of the H_2O_2 is $1.8 \times 10^{-7}M$. In addition, the activity and stability of the films suggest their future applications in the third-generation biosensors.

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