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# An Electrochemical Study of Myoglobin Entrapped in Three Kinds of Films

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*In this paper, we propose a general principle that linear molecules with both hydrophobic and hydrophilic regions may have the capability to enhance the electron transfer reactivity and the catalytic activity of a protein. We have also employed three different kinds of film materials, poly(α,β [N-(2-hydroxyethyl)-DL-aspartamide]), lipoteichoic acid, and 1,12-diaminododecane, in this work for the study of myoglobin to illustrate our findings.*

**Keywords:** Protein Electrochemistry, Myoglobin, Protein-Film Voltammetry, Electroanalysis.

## 1. INTRODUCTION

More and more efforts have been made to achieve the direct electrochemistry of a protein due to the potential applications in various fields, especially biosensors.[1–9] A relatively new technique to realize the direct electrochemistry of a protein is to entrap the protein in some kinds of films, which are later modified on electrode surface. So, increasingly reports are proposed by using various kinds of film materials, such as insoluble surfactants,[10–15] lipids,[16–19] biopolymers,[20–28] etc. But, so far, there is not any in-depth discussion about why and how these kinds of membranes can facilitate the electron transfer between a protein and electrode. In this paper, we take the study of myoglobin (Mb) as an example to summarize our previous works, generalize the unique properties of the film materials. We also report our studies of Mb in membranes of poly(α,β[N-(2-hydroxyethyl)-DL-aspartamide]) (PHEA), lipoteichoic acid (LTA), and 1,12-diaminododecane (DAD), which have not been employed previously, as examples of such kind of research for discussion.

## 2. EXPERIMENTAL DETAILS

### 2.1. Reagents and Chemicals

Horse heart Mb (MW 17,800), PHEA (MW 5,000–20,000), LTA, and DAD were obtained from Sigma. $H_2O_2$ (30% (w/v) solution, analytical grade) was from Nanjing Chemical Reagent Co. They were used without further purification. Other chemicals were all of analytical grade. All solutions were prepared by double distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >18 MΩ cm and stored in the refrigerator at the temperature of 4 °C when not in use.

### 2.2. Preparation of Mb-Film Modified Electrodes

Prior to coating of the protein-films, the substrate pyrolytic graphite (PG) disk 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. Finally, the electrode was thoroughly washed by ultrasonicating in both ethanol and double distilled water for about 5 min. To obtain the best electrochemical response, the experimental conditions for film casting, such as the concentration of Mb and the film materials, the volume ratio of the protein and the film materials, and the total volume of the solutions of the two species, were optimized. Typically, 20 μl of the solution containing $2.8 \times 10^{-5}$ M Mb and 1.2 mg ml$^{-1}$ PHEA, or 1.5 mg ml$^{-1}$ LTA, or 0.8 mg ml$^{-1}$ DAD were spread evenly onto a freshly abraded PG electrode. A small bottle was fit tightly over the electrode so that water was evaporated slowly and more uniform films were formed. The modified electrodes were then dried overnight in air.

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### 2.3. Measurements

Electrochemical experiments were carried out with a Model 283 Potentiostat/Galvanostat (Princetin Applied Research, USA) and a three-electrode system. The working electrode was the modified PG electrode. A saturated calomel electrode (SCE) was used as the reference electrode and all potentials reported here were referred to it. A platinum wire electrode served as the counter electrode. The buffer solutions were purged with purified nitrogen for at least 10 min, and then a nitrogen blanket was maintained during the experiments.

## 3. RESULTS AND DISCUSSION

Mb is a relatively small (Mr 16, 700), oxygen-binding protein of muscle cells. Unlike electron transfer proteins, natively it functions only to store oxygen and to facilitate oxygen diffusion in rapidly contracting muscle tissue. Therefore, no evidence for electron transfer has been found for Mb in solution (Fig. 1(a)). In fact, electrochemical response of Mb in solution has been observed only for solutions prepared by extremely pure Mb.[7–9] However, when Mb is embedded in some types of films, the electron transfer rates can be enhanced greatly.[10–14, 16, 20–23] In this paper, we propose that these film materials have a common property, which is that most of them are linear molecules with a hydrophobic part and a hydrophilic part. To illustrate this point, we have used three different kinds of film materials, PHEA, LTA, and DAD in this work (their chemical structures have been shown in Scheme 1), in addition to the previous reported materials. The $k_s$ values, which can be calculated by the following equation $k_s = \alpha nFv/RT$ where the symbols have the normal meanings, for the three Mb-film modified electrodes are in a relatively large magnitude of order, showing that electron transfer between the electrode and Mb is greatly facilitated in all the three films (Table I) and these film materials have

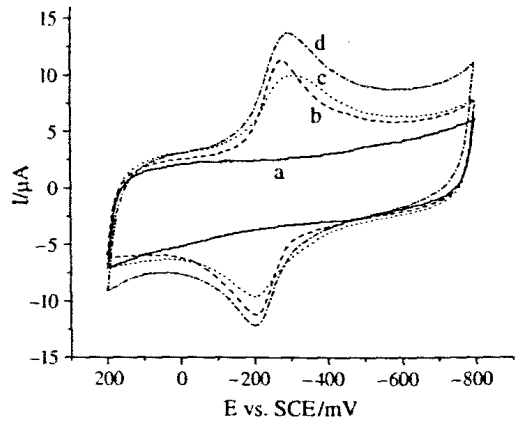
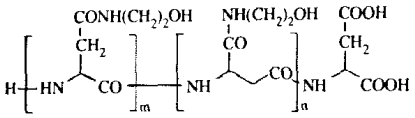
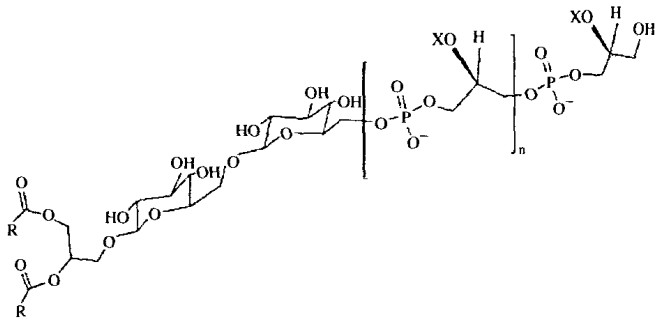


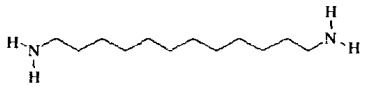

**Fig. 1.** (a) Cyclic voltammogram for 0.04 mM Mb in pH 6.0 phosphate buffer obtained at the bare PG electrode, (b–d) are separately the voltammograms for a protein-free buffer obtained at Mb-PHEA film, Mb-LTA film, or Mb-DAD film modified electrodes. Scan rate: 200 mV $s^{-1}$.



A. poly(α,β[N-(2-hydroxyethyl)-DL-aspartamide])



B. lipoteichoic acid



C. 1,12-diaminododecane

**Scheme 1.** The chemical structures of (A) PHEA, (B) LTA, and (C) DAD.

provided a favorable microenvironment where the protein and the electrode can exchange electron more easily.

The electrochemical behavior of Mb entrapped in the three film materials (PHEA, LTA, and DAD) has been studied by cyclic voltammetry. For a pH 6.0 phosphate buffer, a pair of well-defined, quasi-reversible cyclic voltammogram (CV) peaks can be observed for all the three Mb-film modified electrodes. The formal potentials are about −241 mV, −255 mV, and −249 mV for the protein in PHEA, LTA, and DAD film, respectively (Fig. 1). So, the peaks are located at the potentials characteristic of the heme $Fe^{III}/Fe^{II}$ redox couple of the protein.[10, 29–31] The electrochemical parameters of Mb-PHEA, Mb-LTA, and Mb-DAD film modified electrodes are listed in Table I for comparison. CVs of the three Mb-films modified electrodes have shown nearly symmetrical peak shapes and roughly equal reduction and oxidation peak currents. The reduction peak currents increase linearly with scan rates in the range from 50 to 1000 mV $s^{-1}$. Integration of reduction peaks gives nearly constant charge ($Q$) values with different scan rates. All these results are characteristic of quasi-reversible, surface-confined, and thin-layer electrochemical behavior.[32]

The influence of pH on the voltammetry of the three protein-film electrodes has been examined. Both reduction and oxidation peak potentials of the heme $Fe^{III}/Fe^{II}$ redox

**Table I.** Electrochemical parameters of Mb-film modified electrodes estimated by CV at pH 6.0.

| Film | Deposited $\Gamma$/(mol $cm^{-2}$) | Electroactive $\Gamma^*$/(mol $cm^{-2}$) | $E^{o'}$/mV, vs. SCE | $\Delta E_p$/mV | $k_s/s^{-1}$ |
|---|---|---|---|---|---|
| Mb-PHEA | $95.3 \times 10^{-11}$ | $10.2 \times 10^{-11}$ | −241 | 74 | 84 |
| Mb-LTA | $104 \times 10^{-11}$ | $9.7 \times 10^{-11}$ | −255 | 102 | 77 |
| Mb-DAD | $178 \times 10^{-11}$ | $8.5 \times 10^{-11}$ | −249 | 94 | 74 |

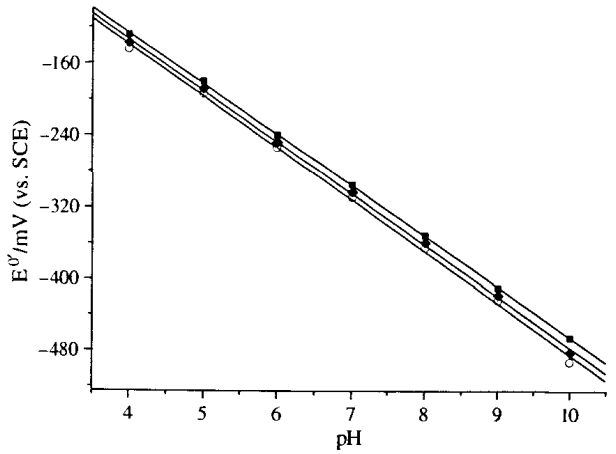



**Fig. 2.** Linear fitting of $E^{o'}$ versus pH for Mb-PHEA film (■), Mb-LTA film (○), and Mb-DAD film (♦). Others same as in Figure 1.

couples for the three Mb-film modified electrodes shift negatively with the pH increase. The formal potential ($E^{o'}$) has a linear dependence on pH in the range of pH 4.0 to 10.0 with a slope of –56.4 mV $pH^{-1}$ for Mb-PHEA, –57.6 mV $pH^{-1}$ for Mb-LTA, and –57.2 mV $pH^{-1}$ for Mb-DAD (Fig. 2). These values are reasonably close to the theoretical values of –58 mV $pH^{-1}$ at 20 °C for a one-proton coupled, reversible single electron transfer, indicating that a single protonation accompanies the electron transfer of Mb $Fe^{III}$ to electrode.[33]

We have also studied the electrochemical catalytic activities of the protein embedded in the three films toward hydrogen peroxide ($H_2O_2$). Taking Mb-DAD as an example, when $H_2O_2$ is added to the buffer, an increase in the Mb $Fe^{III}/Fe^{II}$ reduction peak is observed, accompanied by disappearance of the Mb $Fe^{II}$ oxidation peak (Fig. 3). According to the reaction mechanism, the catalytic procedures can be explained as follows:[34,35]

$$\mathrm{Mb\,Fe(III)} + \mathrm{e} \rightarrow \mathrm{Mb\,Fe(II)}$$

$$\mathrm{Mb\,Fe(II)} + \mathrm{H_2O_2} + 2\mathrm{H^+} \rightarrow \mathrm{Compound} + 2\mathrm{H_2O} + \mathrm{O_2}$$

$$\mathrm{Compound} + 2\mathrm{e} \rightarrow \mathrm{Mb\,Fe(II)}$$

$$\mathrm{Mb\,Fe(II)} - \mathrm{e} \rightarrow \mathrm{Mb\,Fe(III)}$$

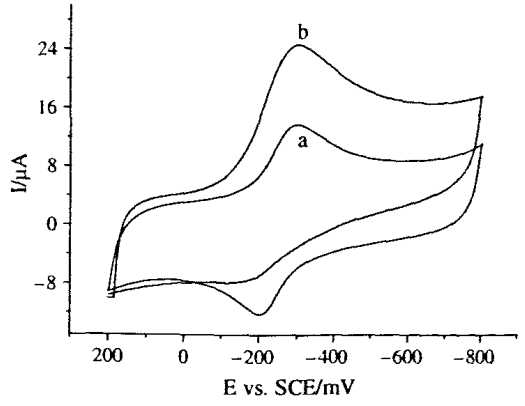



**Fig. 3.** Cyclic voltammograms obtained at Mb-DAD film modified electrode for pH 6.0 phosphate buffer containing (a) no $H_2O_2$; (b) 0.2 mM $H_2O_2$. Others same as in Figure 1.

Same as the other film materials we have employed before, the reproducibility of the response of the Mb-film modified electrodes prepared with these three materials is also very well, and the stability of all the three Mb-film modified electrodes can be satisfactory. Therefore, third-generation biosensors may be consequently developed.

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