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Article

# Electron Transfer Reactivity and the Catalytic Activity of Hemoglobin Incorporated in Dimethylaminoethyl Methacrylate Film

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Hemoglobina (Hb) foi incorporada em Dimetilaminoetil metacrilato (DMAEMA), na preparação de um filme e este foi modificado em eletrodo de grafite pirolítico (PG). O espectro de UV-Vis sugeriu que a hemoglobina no filme manteve sua estrutura secundária. Conseqüentemente, foram observados um par de picos voltamétricos cíclicos, estáveis, bem definidos e *quasi*-reversíveis, com o potencial formal de –206 mV (*vs.* eletrodo de calomelano saturado), característico do par redox Fe(III)/Fe(II) do grupo heme da proteína. A constante de velocidade aparente da transferência de elétron heterogênea e outros parâmetros eletroquímicos foram apresentados. A atividade catalítica da Hb em filmes de DMAEMA, frente ao peróxido de hidrogênio, foi também investigada.

Hemoglobin (Hb) was incorporated in dimethylaminoethyl methacrylate (DMAEMA) to form a film and the film was modified on pyrolytic graphite (PG) electrode. UV-Vis spectra suggested that Hb in the film could keep its secondary structure. Consequently, a pair of stable, well-defined, and quasi-reversible cyclic voltammetric peaks could be observed with the formal potential at –206 mV (vs. saturated calomel electrode), characteristic of heme Fe(III)/Fe(II) redox couple of the protein. The apparent heterogeneous electron transfer rate constant and other electrochemical parameters were presented. The catalytic activity of Hb in DMAEMA films toward hydrogen peroxide was also investigated.

**Keywords:** direct electrochemistry, hemoglobin, dimethylaminoethyl methacrylate, electrocatalysis

## Introduction

Studies on the direct electron transfer between proteins or enzymes and electrode surfaces have showed great significance both in theory and practice.[1-4] However, methods to achieve the direct electron transfer reactions are still limited. Recently, studies have revealed that by incorporating proteins or enzymes in some kinds of films, direct electrochemistry of the macromolecules might be achieved. Lots of laboratories and ours have examined many kinds of film materials to incorporate proteins or enzymes and to facilitate the electron transfer reactivity.[5-25] However, there is still a lot of work to do to try some other materials.

Our previous work has showed that quite a few cationic compounds and their polymers can form fine film structure on electrodes, which is suitable for studying the direct electrochemistry and electrocatalytic behavior of proteins or enzymes. Dimethylaminoethyl methacrylate (DMAEMA) is a colorless, transparent and liquid cationic compound, which can be dissolved in water and many organic solvents, such as ester, ketone, alcohol, ether, hydrocarbon, chlorohydrocarbon, with an amino-like smell. It is an amino-alkaline molecule with a tri-amino, an ester group and unsaturated double-bonds. The tri-amino group can react with acids or alkylating agents such as chloromethane to form salts or quaternary ammonium salts and the ester group can experience hydrolysis or transesterification, while the unsaturated double-bond can undergo addition, polymerization or combined polymerization reaction. Its structure can be described in Scheme 1. DMAEMA can also undergo a series of simple reactions to be artificially synthesized as different polymeric complexes, including novel types of molecules at nano level. Meanwhile, some literatures have revealed that DMAEMA can catalyze the first step of some redox process.[26] Therefore, there is great possibility for DMAEMA to become a new film material which can be used to study direct the electron transfer reactivity

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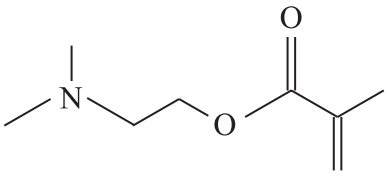
and electrochemical catalytic properties of proteins or enzymes.



**Scheme 1.** Structure of dimethylaminoethyl methacrylate.

## Experimental

*Reagents and apparatus*

Hemoglobin (Hb) was purchased from Sigma. Water was purified with Mill-Q purification System (Barnstead, USA), to a specific resistance > 16 MΩ $cm^{-1}$. Citric acid-sodium citrate buffer (pH 3.0, pH 3.5), HAc-NaAc buffer (pH 4.0 ~ pH 5.5, 0.5 pH interval), PBS buffer ($Na_2HPO_4$-$NaH_2PO_4$, pH 6.0 ~ pH 8.5, 0.5 pH interval), and glycine-NaOH buffer (pH 9.0, pH 9.5, pH 10.0) were all prepared according to literatures. Hb solution (8 mg $mL^{-1}$) was prepared by dissolving 8 mg Hb in 1mL of $H_2O$ and kept at 4 °C. DMAEMA solution was prepared by diluting 5 $\mu$L DMAEMA to 1 mL with pure water.

*Electrode preparation and modification*

The home-made PG disk electrode was prepared by pressing a pyrolytic graphite (PG) rod into a thin glass tube, then fixing it with epoxy resin. A copper wire, which was used to make electrical contact, was fused with the PG rod with the help of wood alloy. The PG electrode was first polished with rough and fine sand paper and then polished to mirror smoothness with an alumina (particle size of about 0.05 $\mu$m)/water slurry on silk. After that, it was rinsed thoroughly and ultrasonicated in bath water for 5 min.

The Hb solution was mixed with an equal volume of DMAEMA solution. 60 $\mu$L of the mixture was dropped onto the electrode surface, and then the modified electrode was dried at room temperature overnight. It was rinsed thoroughly with pure water before use.

*Electrochemical measurements*

Electrochemical experiments were performed on a PAR 263 Potentiostat/Galvanostat (EG&G, USA) with a three-electrode system in a thermostated electrolytic cell at 25±0.5 ºC. A saturated calomel electrode (SCE) and a platinum electrode served as the reference and counter electrode, respectively. The working solution should be deoxygenized with high purity nitrogen prior to detection. A stream of nitrogen was then blown gently across the surface of the protein solution in order to make the solution anaerobic throughout the experiment.

The UV-vis spectrum was obtained with a UV-1601 spectrophotometer (Shimadzu, Japan) in the range from 315 nm to 465 nm.

## Results and Discussion

As is well known, the Soret band in UV-Vis spectra of the protein can provide structural information about Hb. Figure 1a shows that the Soret band of Hb is located at 405.6 nm, consistent with literatures. The band will shift or disappear if the protein structure has been changed and the protein is denatured. As we may notice from Figure 1b, the Soret Band of Hb is located at 406.7 nm after being mixed with DMAEMA solution, with only 1.1 nm shift. So, the structure of Hb entrapped in DMAEMA film has been slightly changed, but not distinctly denatured and the microenvironment around the heme sites of Hb nearly keeps native. Therefore, from our experience, we think that DMAEMA might be a fine film material to be applied to study the direct electrochemistry of the protein.

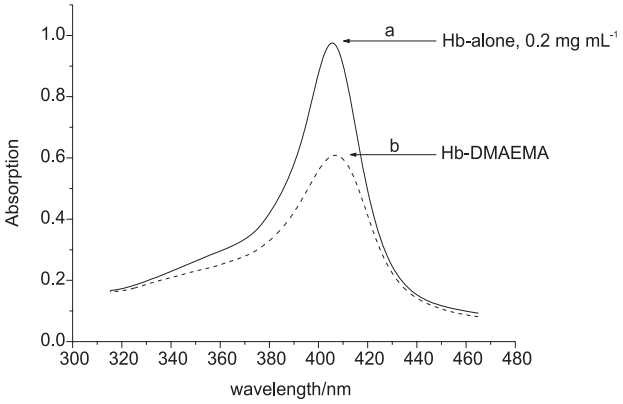



**Figure 1.** UV-Vis spectra for (a) 0.2 mg $mL^{-1}$ Hb solution and (b) Hb-DMAEMA mixed solution.

Figure 2a shows that Hb alone modified PG electrode cannot exhibit any electrochemical response. However, a pair of fine redox peaks can be observed after Hb-DMAEMA co-modified electrode is scanned in the same scan potential range. The anodic and cathodic peaks are -160 mV and -252 mV, respectively. Further studies reveal that DMAEMA alone cannot exhibit any electrochemical signal either. Nor is the bare PG electrode. Therefore the electron transfer reactivity of the protein has been greatly enhanced by DMAEMA after Hb is entrapped in the film. Thus, DMAEMA film should have provided a suitable microenvironment for the protein to take electron transfer

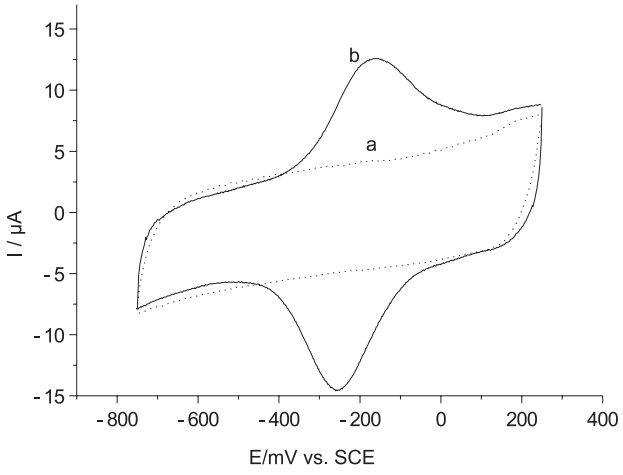



**Figure 2.** Cyclic voltammograms obtained at (a) an Hb modified PG electrode and (b) an Hb-DMAEMA co-modified PG electrode with a pH 5.0, 0.1 M NaAc-HAc buffer. Scan rate: 200 mV $s^{-1}$.

reactions. The mechanism is not clear yet, probably, it is related to the good biocompatibility of the material and the ability of catalyzing the first step of some redox processes.[26]

Figure 3 displays that the both the anodic and cathodic peak currents increase with the scan rate, and the linear

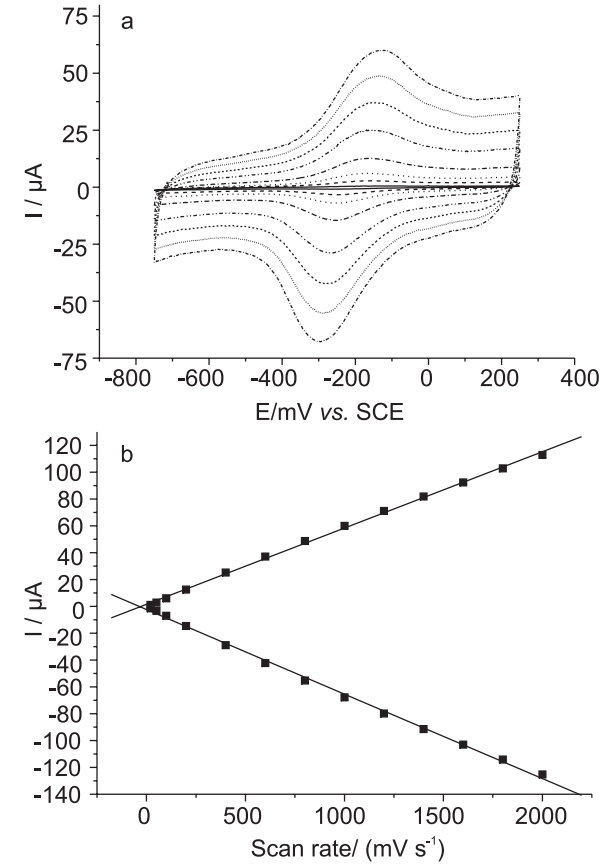



**Figure 3.** (a) Cyclic voltammograms of Hb-DMAEMA co-modified electrode at different scan rate: 10, 50, 100, 200, 400, 600, 800, 1000 mV $s^{-1}$; (b) plot of the peaks currents *vs* scan rate. Others same as Figure 2.

plots can be obtained ranging from 10 mV $s^{-1}$ to 1000 $mVs^{-1}$, as expected for a thin-layer electrochemical behavior.[27] Calculated from Figure 3a, the width at half height ($\Delta E_{p,1/2}$) remains nearly constant for both the cathodic and anodic peaks in the scan range and equal to 108 mV and 115 mV, respectively. And the peak separations ($\Delta E$) also remain constant. These phenomena indicate that the CV responses arise from the surface-confined species.[28, 29] As to the number of electrons (n), it can be estimated to be one from the theoretical value for a quasi-reversible electron reaction, $\Delta E_{p,1/2} = 90/n$ (T = 20 °C), which means that the electron transfer reaction occurring between Hb and the electrode surface was a single-electron transfer process.

The surface coverage of the electroactive substance ($\Gamma^*$) has also been calculated according to Faraday's law:

$$\Gamma^* = Q/nFA \quad (1)$$

$\Gamma^*$ stands for adsorptive quantity on electrode surface, $Q$ stands for peak area (quantity of charge) and $A$ was the electrode area. Since $A$ is 0.06 $cm^2$ and $Q$ is $8.726 \times 10^{-6}$ C when the scan rate is 200 mV $s^{-1}$, the surface coverage of the electroactive protein can be known as $2.06 \times 10^{-9}$ mol $cm^{-2}$. So, DMAEMA has well maintained the electrochemical reactivity of the protein.

$\Delta E$ keeps less than 200 mV, as scan rate varies from 10 mV $s^{-1}$ to 1000 mV $s^{-1}$. When $n\Delta E < 200$ mV, the electrode transfer rate could be calculated by the following equation.[8]

$$Ks = \alpha nFv/RT \quad (2)$$

So, the electrode transfer rate ($Ks$) can be calculated as 4.07 $s^{-1}$, when scan rate is 200 mV $s^{-1}$. The average value of Ks is 11.19 $s^{-1}$ at the scan rate ranging from 100 to 1000 mV $s^{-1}$. So, the electron transfer rate in the DMAEMA film can be as high as that in the other good films.[30]

The cyclic voltammograms of the Hb-DMAEMA co-modified electrode have also been recorded in various buffers with different pH values. Experimental results indicate that fine voltammograms can be obtained in a wide pH range (pH 3.0-pH 10.0) in all the test buffers such as citric acid-sodium citrate, HAc-NaAc, PBS and glycine-NaOH buffers. And the influence of pH on the electrochemistry is reversible, since same CV can be obtained if the modified electrode is moved to the original buffer from other solutions.

In the meantime, experimental results have revealed that increase of pH value will result in the negative shift of the standard potential and plot of $E_{1/2}$ *vs* pH indicates a linear correlation within the pH range from 3.0 to 10.0.

The slope ($\Delta E/\Delta pH$) is -48.7 mV $pH^{-1}$ (scan rate: 100 mV $s^{-1}$) and -48.06 mV $pH^{-1}$ (scan rate: 200 mV $s^{-1}$), close to the theoretical value (-59 mV $pH^{-1}$) of a single-proton concomitant single-electron transfer process.

Further studies reveal that the peroxidase acitivity of Hb has also been enhanced after the protein is entrapped in the DMAEMA film. Figure 4 shows the cyclic voltammograms obtained at the Hb-DMAEMA co-modified electrode in the presence and the absence of hydrogen peroxide ($H_2O_2$) in the buffer solution. It can be seen that a new reduction peak appears at -150 mV after $H_2O_2$ is added in the buffer, and the peak increases with the $H_2O_2$ concentration. Since no peak occurs with a DMAEMA alone modified PG electrode, we can infer that the catalytic peak is due to the effect of Hb on $H_2O_2$. Figure 5 shows the relationship between the catalytic peak current and the $H_2O_2$ concentration. A linear plot can be obtained from $2.0\times10^{-5}$ to $3.0\times10^{-4}$ mol $L^{-1}$.

The Hb-DMAEMA film is very stable toward the catalytic activity. The catalytic peak will decrease by less than 5% after exposed to air for two weeks, while it decreases by 10% after 30 days.

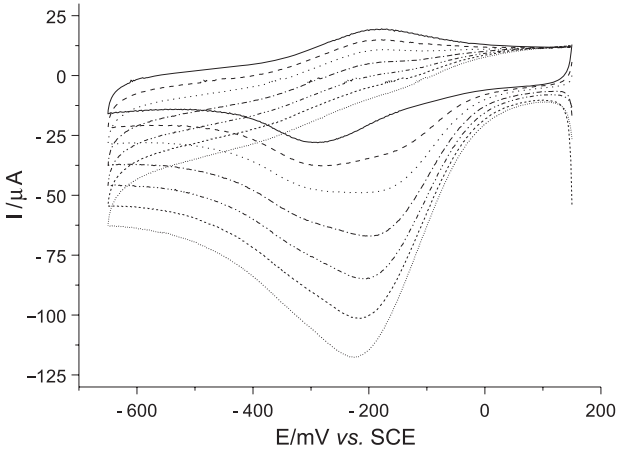



**Figure 4.** Cyclic voltammograms obtained at the Hb-DMAEMA modified electrode in $H_2O_2$ gradient solutions. Other same as Figure 2.

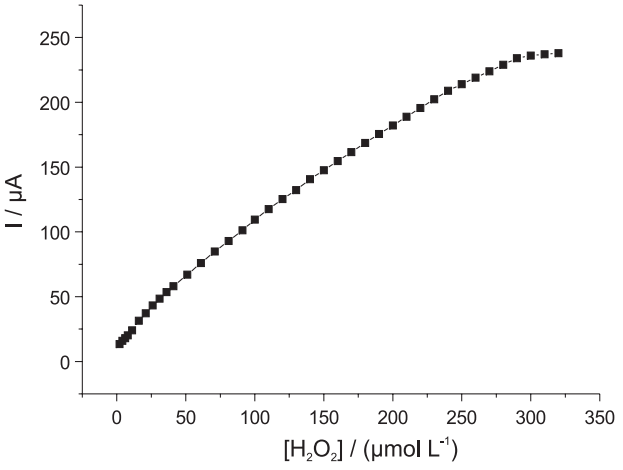



**Figure 5.** Plot of catalytic peak current *vs* $H_2O_2$ concentration. Other same as Figure 2.

## Conclusions

By incorporating Hb in DMAEMA films successfully and modifying them on electrode surfaces, the direct electron transfer rate between Hb and the electrode was enhanced. Certain electrochemical parameters of the Hb-DMAEMA film were also obtained with CV. The Hb-DMAEMA film had certain catalyze ability on hydrogen peroxide, which is the base for developing electrochemical sensors in the future.

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