Lipid Film에 수식된 헤모글로빈의 전기화학적 특성과 H_2O_2 응답특성

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Direct electrochemistry of hemoglobin at carbon electrode modified with lipid film and its application as a H_2O_2 sensor

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Abstract - In this research, the enhancement of electron-transfer activity of hemoglobin (Hb) in dodecanoic acid film was investigated for the first time. This type of composite film was made on glassy carbon electrode by casting method. Cyclic voltammetric result of the modified electrode displays a well defined redox peaks which was attributed to the direct electrochemical response of Hb. Our results illustrate that Hb exchange electrons directly with electrode and exhibits the characteristics of peroxidase. When we apply this modified electrode as a biosensor, it gives excellent performances in the electrocatalytic reduction of hydrogen peroxide (H_2O_2). Through the optimal conditions, the proposed biosensor shows the linear range for H2O2 determination was from 1×10^{-5} to 1.25×10^{-4} mol/L with a detection limit of 1×10^{-7} mol/L. The biosensor retained more than 90% of the initial response after 14 days.

1. Introduction

Direct electron transfer of heme-protein has attracted many scientists' interest due to some reasons. Of them, one of the reasons is that it can be used to prepare biosensors. Generally, Hb molecules exhibit a rather slow rate of heterogeneous electron transfer at electrodes because of electroactive center embedded deeply in the protein structure. Therefore, electron transfer of protein can be enhanced by immobilizing them into proper films [1]. Direct electron transfer of Hb has been achieved with some different type of films [2-5]. Since it is very difficult for Hb to perform redox reaction at electrode surface, to realize its electrochemical properties are still many scientists' willness.In this context, to enhance electron transfer ofHb, dodecanoic acid was used in this study. We have examined the electron transfer of Hb after casting the mixture of dodecanoic acid and Hb onto the glassy carbon electrode. To the best of our knowledge, this is the first time that direct electron transfer of Hb has been investigated in dodecanoic acid films. As hemoglobin has similar peroxidase activity [6-8], it can be used to catalyze the reduction of H2O2. Therefore, in this work, an amperometric H2O2 biosensor was developed based on direct electrochemistry of Hb. direction. Amperometric measurements were taken by applying a potential of -300 mV to the working electrode. A magnetic stirrer was used for this experiment. Prior to the experiments, all solutions were purged with high purity nitrogen for at least the 30 minutes.

2. Experimental

(1) Reagents

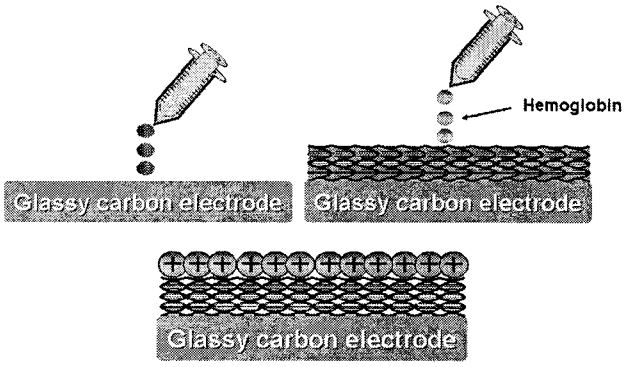
Hemoglobin was purchased from sigma. Hb was used without further purification. Dodecanoic acid and hydrogen peroxide (30 wt%) were obtained from Aldrich. Stock solutions of H_2O_2 were diluted from 30% solution. All other reagents were of analytical grade. The stock solutions were stored at a temperature of 40 C. All experimental solutions were prepared daily by appropriate dilution of the stock solution. All solutions were prepared by using double- distilled water. Water was purified with a Milli-Q purification system.

(2) Intsrumentation and electrochemical measurement

Electrochemical measurements were carried out with CHI (630B) electrochemistry workstation. The three-electrode system was applied for these experiments. A Lipid-Hb modified glassy carbon electrode was used as a working electrode. An Ag/AgCl electrode was employed as the reference electrode and platinum wire was connected as the counter electrode. The stock solutions were prepared by Phosphate Buffer Solution (PBS) with a pH value of 7.0. HCl was used to maintain pH. For cyclic voltammetry, the modified electrode was cycled in PBS (0.1M) by applying voltage in a range between 0.3 to -0.7 V vs. Ag/AgCl in the negative scanning direction. Amperometric measurements were taken by applying a potential of -300 mV to the working electrode. A magnetic stirrer was used for this experiment. Prior to the experiments, all solutions were purged with high purity nitrogen for at least the 30 minutes.

(3) Electrode preparation

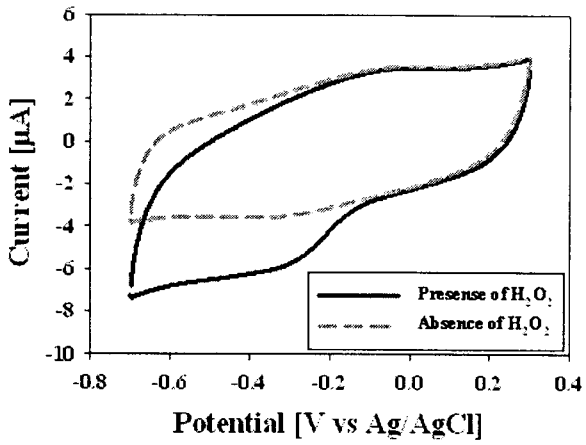
At first, the electrode was cleaned alumina Then, the electrode was rinsed with ethanol and pure water before modification. Just after washing, 5 μl of dodecanoic acid (2 mg/ml) and 5 μl of 0.1 mM Hb alternately were deposited onto the electrode surface by droplet method. The electrode was dried overnight at room temperature. The modified electrode was stored in phosphate buffer solution with pH 7.0 at 40 ℃ in the refrigerator when it was not in use. Fig 1 shows the schematic diagram of electrode fabrication.



<Fig.1> Schematic diagram of electrode fabrication.

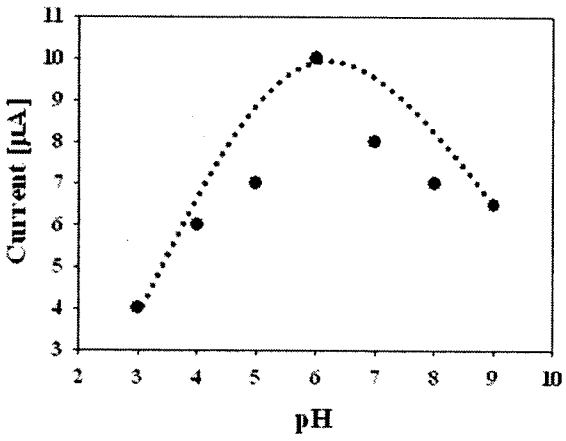
3. Results and Discussion

The cyclic voltammogram of Lipid/Hb modified glassy carbon electrode is shown in Fig. 2. A pair of well defined redox peaks was observed in CVs of Lipid/Hb-modified electrode (solid line).No peak was observed at bare or only lipid modified electrode. This result indicates that the redox peaks arise from the Hb embedded in the lipid film. It also illustrates that the good biological compatibility of lipid (dodecanoic acid) film enhance the electron transfer rate between Hb and electrode. The observed cathodic and anodic peak potentials are located around at -280 mV and -90 mV, respectively, at a scan rate of 50 mV/s. The effect of scan rate on the response of the Lipid/Hb modified electrode was found that with the increase of scan rates (from 50 to 250 mV/s), the redox peak currents of the Hb increased linearly (data not shown here). The experimental data proves that this redox process is quite quasi-reversible and surface controlled. This figure also shows the cyclic voltammograms of Lipid/Hb modified electrodes before and after added of H_2O_2. The cathodic peak current is increased by the addition of H_2O_2 while the anodic peak current is decreased, that indicates an electrocatalytic reduction process of H_2O_2. This obvious increase in the cathodic current also illustrates that the Hb keeps its natural structure after immobilizing with Lipid onto the carbon electrode. In this context, it also can be said that lipid plays an important role in preventing the protein denaturation at the electrode surface.



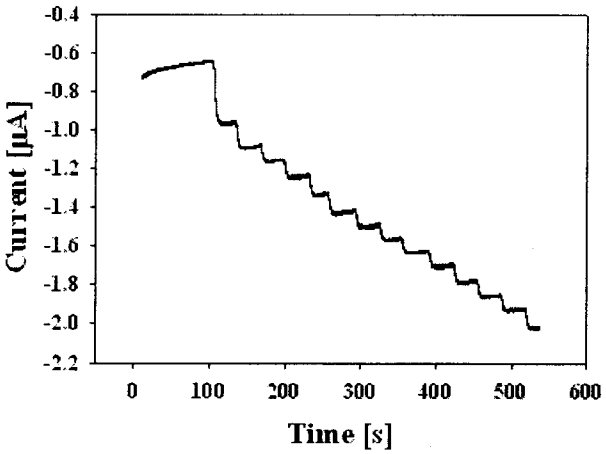
<Fig. 2> Cyclic voltammogram of lipid/Hb modified electrode in the presence of H_2O_2 (dashed line) and absence of H_2O_2 (solid line).

The influence of applied potential on amperometric response of the biosensor is also an important parameter. Therefore, to optimize the applied potential for H_2O_2 detection, we investigated the effect of applied potential on the response current. In this experiment, we observed the response current of the sensor at different applied potential in the presence of 2×10^{-5} M H_2O_2. According to this experiment, the maximum current was achieved in a potential range of -200 to -300 mV. At more negative potential, there may be some risk of an interfering reaction by the other electroactive species in the solution. Therefore, applied potential of -300 mV was chosen as the working potential for this sensor. We examined the current response of the sensor in the pH range of 3.0 to 9.0. According to the experimental results, PBS with pH 6.0 was chosen as the supporting electrolyte for the biosensor in order to achieve maximum sensitivity and good bioactivity. (Fig. 3)



<Fig. 3> Effect of the pH Containing the solution of 0.4 mM H_2O_2 at modified Electrode

Fig. 4. shows the amperometric response of the Lipid/Hb modified electrode under optimal conditions where the potential was kept at -300 mV in 0.1 M PBS with pH 6.0. It is confirmed that after successive addition of 1×10^{-5} M H_2O_2, a well defined response is observed. In the case, each injection of H_2O_2 within a response time of calculation less than 5 second a sharp increase of current was observed. Through the optimal conditions, the proposed biosensor shows the linear range for H_2O_2 determination was from 1×10^{-5} to 1.25×10^{-4} mol/L with a detection limit of 1×10^{-7} mol/L. The biosensor retained more than 90 % of the initial response after 14 days.



<Fig. 4> Typical Current-time response of the sensor with successive additions of 1×10^{-5} H_2O_2 at the 0.1 M PBS.

4. Conclusion

We investigated the direct electron transfer of Hb in lipid film. Our result indicates that lipid can make a suitable microenvironment for Hb to enhance electrons transfer onto the glassy carbon electrode. It was also found that Hb keeps its native structure after immobilization with lipid onto the electrode. By applying as a biosensor, this sensor shows an excellent catalytic activity towards H2O2.Ease of fabrication, quick response and high stabilty are the particular advanatges of this sensor.

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