Hemoglobin-DNA/pyterpy 박막을 이용한 과산화수소의 전기화학적 검출

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Electrochemical Detection of Hydrogen Peroxide based on Hemoglobin-DNA/pyterpy Modified Gold Electrode

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Abstract - Hydrogen peroxide (H_2O_2) biosensor is one of the most developing sensors because this kind of sensors is highly selective and responds quickly to the specific substrate. Hemoglobin (Hb) has been used as ideal biomolecules to construct hydrogen peroxide biosensors because of their high selectivity to H_2O_2. The direct electron transfer of Hb has widely investigated for application in the determination of H_2O_2 because of its simplicity, high selectivity and intrinsic sensitivity. An electrochemical detection for hydrogen peroxide was investigated based on immobilization of hemoglobin on DNA/Fe(pyterpy)$^{2+}$ modified gold electrode. The pyterpy monolayers were firstly an electron deposition onto the gold electrode surface ofthe quartz crystal microbalance (QCM). It is offered a template to attach negatively charged DNA. The fabrication process of the electrode was verified by quartz crystal analyzer (QCA). The experimental parameters such as pH, applied potential and amperometric response were evaluated and optimized. Under the optimized conditions, this sensor shows the linear response within the range between 3.0×10^{-6} to 9.0×10^{-4} M concentrations of H_2O_2. The detection limit was determined to be 9×10^{-7} M (based on the S/N=3).

1. Introduction

Since the first enzyme-based biosensor, invented by Clark and Lyons [1], there has been growing interest in the field of biosensors. A biosensor is usually an analytical device that consists of bioreceptor, transducer and target analyte. It is typically used in biotechnology, medicine, industries and environmental monitoring [2]. The detection of hydrogen peroxide (H_2O_2) is of great interest to biochemists because of its importance in the pharmaceutical, clinical and industrial settings. There are many research groups directing their efforts to determine H_2O_2 with low cost as well as within the short time.

Electrochemical methods, such as amperometric biosensors based on simple and economical immobilized redox proteins or enzymes modified electrodes, have been widely employed for determination of H_2O_2 for their simplicity, high selectivity and intrinsic sensitivity. For these biosensors, horseradish peroxidase (HRP) is the most commonly used enzyme. But the HRP is not very stable in solution and is very expensive. In this point of view, HRP is the most commonly used enzyme [3]. Alternatively, hemoglobin is one of the promising bio-molecules that can be used as a bioreceptor element. Hemoglobin, the main component in red blood cells, is a kind of protein that picks up oxygen in the lungs and delivers it to the body tissues. It has a molar mass of 67,000 g/mol and consists of four kinds of electro-active iron hemes. In contrast to most other proteins, Hb can give long-term stability to the H_2O_2 sensor as compared to other proteins. In addition, Hb is cheaper than the commonly used HRP [2].

Immobilization of protein molecules in biocompatible films or matrix, especially in DNA films, have attracted much attention [4] since Nassar and Rusling reported DNA can enhance electron transfer between electrode and heme proteins in myoglobin-DNA films [5]. DNA is a substance that has a three dimensional structure. It is not only a biological macromolecule but also a conductive polymer, because DNA stacked base pairs can be considered as a system of connecting electrode to transfer electrons. Incorporation of Hb in the DNA based films enhance the Hb electron transfer rate [6].

In this study, a hydrogen peroxide biosensor was fabricated by immobilizing Hb and DNA on Fe(pyterpy)$^{2+}$/Au electrode which is very simple and convenient using self-assembly and layer by layer method. To the best of our knowledge, for the first time, we have designed a hydrogen peroxide biosensor based on Hb-DNA modified gold electrode where the pyterpy monolayers have been performed a very important role for enhancing electrons transfer rate of Hb-DNA. The formed DNA film worked as providing a compatible micro-environment for the protein and a direct pathway of electron transfer between Hb and the electrode surface. This biosensor exhibits a quick response and high stability to the H_2O_2.

2. Experiment

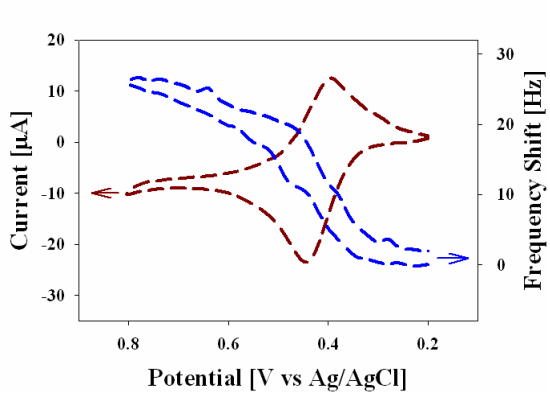
$Fe(pyterpy)_2(BF_4)_2$ was synthesized by Qian et al. [7]. Hemoglobin was obtained from Sigma. Their stock solutions were stored at a temperature of 4℃. Double strand DNA was purchased from Bioneer Corporation (Daejeon, Korea). Stock solutions of H_2O_2 were diluted from 30% solution. All other reagents were of analytical grade. Hb and DNA were used without further purification. The experimental solutions were prepared everyday by appropriate dilution of the stock solution. All the stock solutions were prepared fresh with distilled water which was purified with a Milli-Q purification system.

The pyterpy monolayers were modified on the gold electrode of QCM. Prior to use, the gold electrode of QCM was cleaned by piranha solution (H_2SO_4:H_2O_2=3:1) subsequently cleaned by cycling potential windows between 0 and 1.5 V in 0.05 M H_2SO_4 solution at a scan rate of 100 mV/s for nearly 25 minutes until stable scans were recorded. Then the electrode was thoroughly rinsed with the distilled water. After pretreatment, the electrode was immersed in chloroform-DMF solution (7:3) with pure Ar gas. Finally, the pyterpy modified electrode was immersed into phosphate buffer solution (PBS) containing 3 mg/ml hemoglobin and DNA for 5 hours. The modified electrodes will be abbreviated as Hb-DNA/Fe(pyterpy)$^{2+}$/Au and Fe(pyterpy)$^{2+}$/Au, respectively.

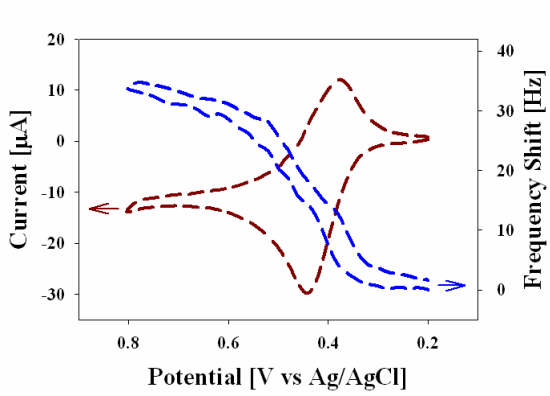
The 9 MHz QCM (AT-cut, 5 mm diameter) which was provided with gold-coated electrode, were obtained from Seiko EG&G (Japan). The measurement system is consisted of electrochemical three electrode set-up using QCA (Seiko EG&G, Japan) and Potentiostat. (PerkinElmer, USA). A three electrode configuration was employed for these experiments. The Hb-DNA/Fe(pyterpy)$^{2+}$ modified gold electrode was used as the working electrode. The Pt wire and KCl saturated Ag/AgCl electrodes were used as counter and reference electrodes, respectively. The stock solutions were made by PBS (pH 6.5).

3. Results and Discussion

The QCM method is a powerful technique for the measurement of nano-scale mass changes that are based on the relationship between resonant frequency shift and mass changes. The measured frequency shifts were about 268 Hz by ten times. From the results, we calculated that the adsorption mass was about 286 ng/cm^2 according to the Sauerbrey equation [8]. So, we can know the assembled amount of Hb-DNA/Fe(pyterpy)$^{2+}$ on gold electrode of QCM.



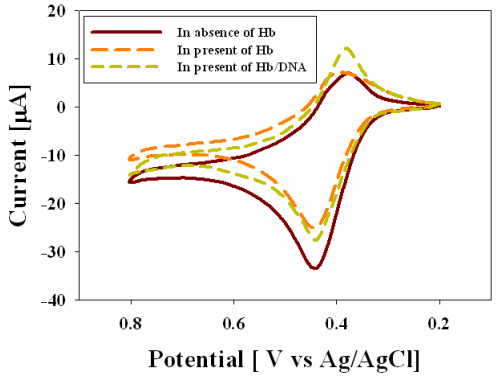
(a) 0.1 M $NaClO_4$



(b) 0.1 M Na_2SO_4

<Fig. 1> EQCM property of $Fe(pyterpy)^{2+}$/Au modified electrode in 0.1 M $NaClO_4$ and Na_2SO_4 electrolyte solution.

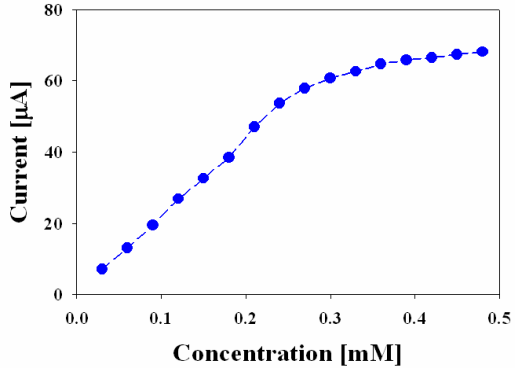
Fig. 1 shows the resonant frequency change which obtains during the cyclic voltammetry (CV). The frequency change was about 11.8 Hz and 14.3 Hz, respectively. From the data, the transferred mass was about 12.6 ng and 15.3 ng by Sauerbrey equation. Multiplying by Avogadro number, the number of shifted ions was about 1.52×10^{13} and 1.84×10^{13} [8].



<Fig. 2> Cyclic voltammograms of $Fe(pyterpy)^{2+}$/Au, Hb/$Fe(pyterpy)^{2+}$/Au and Hb-DNA/$Fe(pyterpy)^{2+}$/Au electrode.

Fig. 2 shows the CVs recorded for the $Fe(pyterpy)^{2+}$/Au, Hb/$Fe(pyterpy)^{2+}$/Au and Hb-DNA/$Fe(pyterpy)^{2+}$/Au electrode. The peaks are located at 380 mV and 440 mV. After the immersion of the $Fe(pyterpy)^{2+}$/Au into the solution containing Hb and Hb-DNA, there are a new pair of peak. It can be stated that new redox peaks arise from heme groups of Hb that immobilized onto the pyterpy modified electrode. Normally only hemoglobin and DNA does not show any electrochemical characteristics as mentioned above. So, in case of this, pyterpy monolayers are playing an important role to exchange electrons between Hb-DNA and electrode.

The pH value is one of the most important factors for working with biosensor that affect the response of the electrode. So, we investigated the pH effect on the sensor response in presence of H_2O_2. According to result, it can be seen that the peak current which attains the maximum level at pH 6.5. Therefore, pH 6.5 was chosen to be used for working media solution to determine H_2O_2 of this sensor.



<Fig. 3> Calibration of the sensor.

Fig. 3 displays calibration plot obtained for the Hb-DNA/$Fe(pyterpy)^{2+}$ modified gold electrode using optimum experimental conditions. Under the optimized experimental parameters, this sensor shows the linear response within the range between 3×10^{-6} to 9×10^{-4} M concentrations of H_2O_2. The detection limit was determined to be 9×10^{-7} M (based on the S/N =3). This detection limit is lower than the other biosensor [9].

4. Conclusion

We have used the self-assembly and layer by layer method to prepare electrode using pyterpy monolayers. The electrochemical property of pyterpy monolayeys was characterized in 0.1 M $NaClO_4$ and Na_2SO_4 electrolyte solutions using cyclic voltammetry. The modified electrode showed reversible property and high stability. The EQCM response was simultaneously determined by resonant frequency during cyclic voltammetry. From the result, the immersed pyterpy monolayers can act as an electron transfer mediator to access to the electrode surface. We have also demonstrated a simple method for designing a H_2O_2 biosensor using Hb-DNA. It has proved that pyterpy provided a suitable biocompatible microenvironment for Hb-DNA. The catalytic properties of the designed sensor proved that Hb-DNA has been kept its natural structure and can retain its biological activity. The designed biosensor shows fast amperometric response, excellent linearity and low detection limit. In addition, it shows very high sensitivity, good reproducibility and stability.

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