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Viologen 유도체를 전하전달체로 이용한 Glucose 센서의 H_2O_2 검출 특성

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H_2O_2 Detection Property of Glucose Sensor using Self Assembled Viologen Modified Electrode as Mediator

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Abstract - An amperometric glucose biosensor has been developed using viologen derivatives as electron mediator of glucose oxidase (GOD) at Au electrode. Highly stable self assembled monolayer (SAM) of thiol-based viologen is immobilized onto the Au electrode followed byGOD is immobilized onto the viologen modified electrode. This biosensor response to glucose was evaluated amperometrically in the potential of -300 mV. Upon immobilization of glucose oxidase onto the viologen modified-electrode, the biosensor showed rapid response towards glucose. Experimental conditions influencing the biosensor performance such as, pH, potential were optimized and assessed. This biosensor offered an excellent electrochemical response for glucose concentration below μmol level with high sensitivity and selectivity and short response time. The levels of the RSD's (< 5 %) for the entire analyses reflected the highly reproducible sensor performance. Using the optimized a linear relationship between current and glucose concentration was obtained up to 4.5×10^{-4} M. In addition, this biosensor showed well reproducibility and stability.

1. Introduction

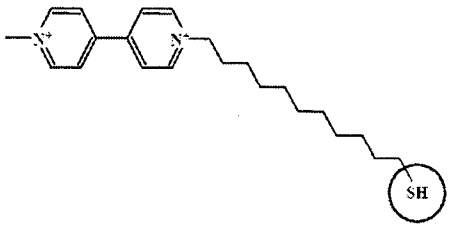
Viologen derivatives have been widely investigated their redox activity and electrochromic properties [1,2]. They are attractive materials because of their chemical stability, their relatively simple behavior of redox reaction and their possible practical applications due to their electrochemical properties. Since Clark and Lyons first proposed the initial idea of glucose enzyme electrodes in 1962, an increasing interest has been paid toward development of the biosensor for glucose measurement [3]. Most of the known glucose sensors are based on the electrochemical oxidation of hydrogen peroxide which is produced from immobilized enzyme with the help of dissolved oxygen [4]. However, the amperometric measurement of hydrogen peroxide oxidation requires a relatively high working potential (over 0.6 V), at which other species such as uric acid and ascorbic acid are also electro-active. Therefore, researchers attempted to minimize errors by interfering electro-active species in glucose sensors. In the case of this the consumption of oxygen by mediator can be used to design more sophisticated glucose sensor.

In this work, thiol modified viologen has been used to design glucose biosensor as redox mediator. Viologen ($VC_{10}SH$) play an important role as electron relays in systems in which electron transfer is initiated by electrochemical process [5]. It exhibits fast reversible electrochemical characteristics at negative potentials that can make it useful as redox mediators for enzymatic reaction [6]. In this present work, glucose oxidase (GOD) was immobilized onto the thiol-functionalized self assembled viologen modified gold (Au) electrode. It is expected to increase the efficiency of electron transport and the sensitivity of the GOD modified electrode by viologen as electron mediator. The sensor based on this, exhibits excellent performance, a fast response, nice sensitivity, and low detection limit.

2. Experimental

2.1 Reagents

Viologen derivative ($VC_{10}SH$) was synthesized by Dr. Qian (Fudan University, China). Fig. 1 shows the molecular structure of the viologen bonded with a thiol group. The rest of the reagents used in this experiments were of analytical grade and used without any purification. All solutions were prepared using Milli-Q water. Glucose oxidase and glucose were purchased from Sigma. Their stock solutions were stored at a temperature of 40C. All other reagents were of analytical grade. The experimental solutions were prepared everyday by appropriate dilution of the stock solution. All the stock solutions were prepared fresh with distilled water. Water was purified with a Milli-Q purification system.



<Fig. 1> Molecular structure of viologen derivative.

2.2 Electrode modification

At First, the gold electrode was cleaned by piranha solution (H_2SO_4:H_2O_2=3:1) solution subsequently cleaned by cycling between potential windows of 0 to + 1.5 V versus Ag/AgCl 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 water. After pretreatment, the electrode was immersed in an ethanol-acetonitrile (1:1) solution containing 2 mM thiol-functionalized viologen for 24 hours. After self-assembled, the electrode was removed from the deposition solution and rinsed with ethanol and water to remove weakly adsorbed viologen. After then, the viologen-modified electrode was immersed into phosphate buffer solution containing 5 mg/ml glucose oxidase for 5 hours.

2.3 Apparatus and electrochemical measurement

Cyclic voltammetry (CV) and chronoamperometry (CA) were carried out with the CHI 630B workstation. A three electrode configuration was employed for these experiments. The self-assembled viologen monolayer onto 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.

3. Results and Discussion

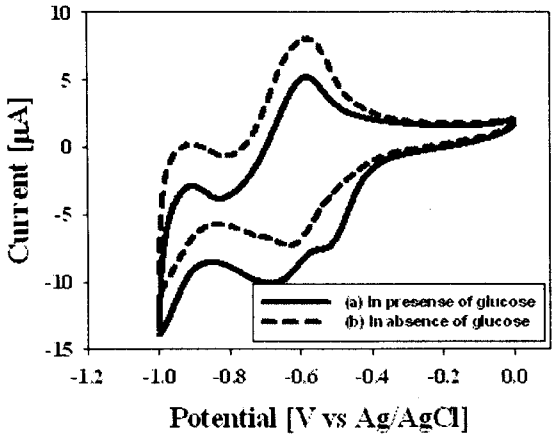
3.1 Electrochemistry of the GOD/Viologen modified gold electrode

The modified electrodes were characterized by cyclic voltammetry to confirm the electron flow from the gold electrode. Fig. 2 (a) shows the voltammogram of the GOD/Viologen/Au electrode in phosphate buffer solution at pH 7.0. The oxygen reduction peak can be observed at about -0.5 V together with typical redox peak of viologen. Fig. 2 (b) shows the voltammo- gram of the GOD/Viologen/Au electrode in the phosphate buffer solution containing 5 mM glucose. Interestingly, in the presence of glucose the reduction peak of oxygen completely disappears. It is assumed that the catalytic effect of the oxygen reduction was occurred by the viologen derivative. The mechanism of the reduction of oxygen by viologen can be described as similar as follows [7,8].

(1) $V^{2+} + e^- \rightarrow V^+$
(2) $V^+ + O_2 \rightarrow V^{2+} + O_2^-$
(3) $2O^{2-} + 2H^+ \rightarrow O_2 + H_2O_2$

In the presence of glucose, there is also the reaction:

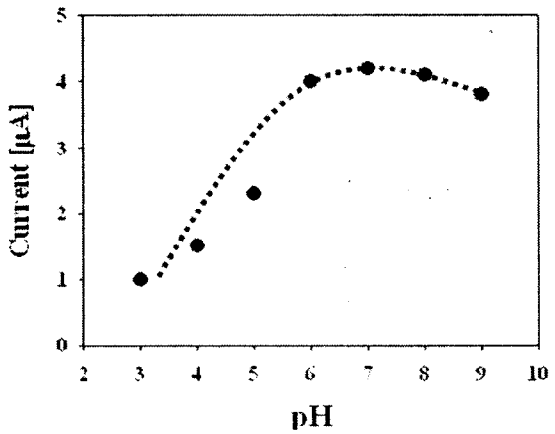
(4) Glucose + O_2 + H_2O → Gluconic acid +H_2O_2



<Fig. 2> GOD/Viologen/Au electrode in the absence and in the presence of 5 mM glucose.

3.2 Optimal condition, selectivity and stability of the biosensor

The effect of the pH in the buffer solution on the biosensor response is one of the most important factors. From this, the pH effect on the sensor response in presence of 5×10^{-5} M glucose in PBS (0.1 M) was investigated. The current response of the biosensor in the pH range of 3.0 to 9.0 was evaluated. Fig. 3 shows the biosensor response for the glucose in various pH ranges. It can be seen that the current response increased from pH 3.0 to 7.0, and attained the maximum current at pH 7.0. In strong basic or strong acidic solution, the biocompounds were denatured. Therefore, PBS with pH 7.0 was chosen as a supporting electrolyte in order to get maximum sensitivity and good bioactivity.



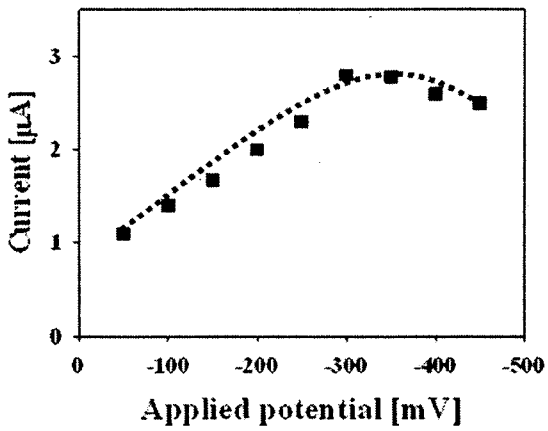
<Fig. 3> Effect of the pH Containing the solution of 5×10^{-5} M glucose in 0.1 M PBS.

Fig. 4 shows the current response of the biosensor in various applied potential. The influence of applied potential on current response of the biosensor in the presence of 5×10^{-5} M glucose was studied. Based on the experimental data, the steady-state current increases gradually as voltage increases from 0 mV to -300 mV. According to this experiment, the maximum current was achieved in the range of -300 mV. At a morenegative potential, there may be some risk of interfering reaction of the other electroactive species in the solution. Therefore, we have chosen applied potential - 300 mV as the working potential for this sensor.

We also investigated the effect of some substances which interfere with the response of the proposed biosensor. The long-term stability of this biosensor was also checked over a 30 day period. To examine this, we checked the response of the Viologen/GOD-modified electrode to the glucose. It retained 80% of its initial current response after 30 days.

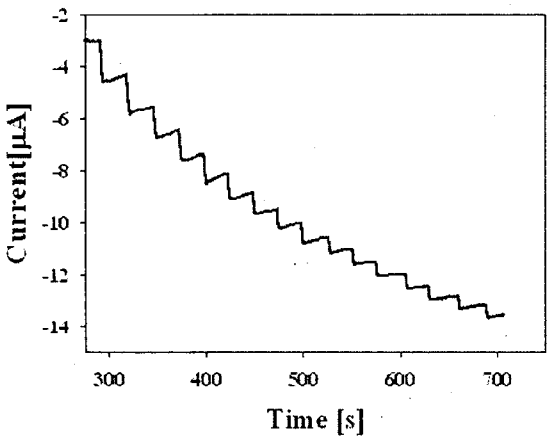
3. 3 Amperometric response of the glucose sensor

Fig. 5 shows the amperometric response of the Viologen/GOD modified Au electrode under optimal conditions where the potential was kept at -300 mV in 0.1 M PBS with pH 7.0. It is confirmed that after successive addition of 1×10^{-5} M glucose, a well defined response is observed. In the case of each injection of glucose within a response time



<Fig. 4> Influence of applied potential on amperometric response of the sensor.

of ca. less than 5 s a sharp increase of current was observed. Through the optimal conditions, the plot of current as a function of glucose concentration shows a linear line in glucose concentration between 3×10^{-5} and 4.5×10^{-4} M with a detection limit of 3×10^{-6} M.



<Fig. 5> Amperometric response at the modified electrode on successive additions of glucose.

4. Conclusion

We designed a simple and promising biosensor for glucose detection, which is consisted of viologen and glucose oxidase. Our results illustrate that GOD exhibits nice bioactivity in viologen-modified Au electrode. The proposed biosensor showed nice sensitivity and stability, even though its detection limit is not enough for practical use. Finally, ease of fabrication, low cost, fast response time, good sensitivity, and stability are obvious advantages for this newly proposed modified electrode.

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