Electrochemical Detection of Self-Assembled Viologen Modified Electrode as Mediator of Glucose Sensor

Dong-Yun Lee*, Won-Suk Choi*, Sang-Hyun Park* and Young-Soo Kwon†

Abstract – An amperometric glucose biosensor has been developed using viologen derivatives as a charge transfer mediator between a glucose oxidase (GOD) and a gold electrode. A highly stable self-assembled monolayer (SAM) of thiol-based viologen was immobilized onto the gold electrode of a quartz crystal microbalance (QCM) and GOD was immobilized onto the viologen modified electrode. This biosensor response to glucose was evaluated amperometrically in the potential of -300 mV. Upon immobilization of the 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 excellent electrochemical responses for glucose concentration below μ mol level with high sensitivity and selectivity and short response time. The levels of the RSDs (< 5 %) for the entire analyses reflected the highly reproducible sensor performance. A linear calibration range between the current and the glucose concentration was obtained up to 4.5×10^{-4} M. The detection limit was determined to be 3.0×10^{-6} M.

Keywords: Glucose Biosensor, Viologen, Mediator, Glucose Oxidase, Detection Limit

1. Introduction

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 [1]. Most of the known glucose sensors are based on the electrochemical oxidation of hydrogen peroxide which is produced from immobilized enzymes with the help of dissolved oxygen [2]. However, the amperometric measurement of the hydrogen peroxide oxidation requires relatively high working potential (more than 0.6 V), at which other species such as uric acid and ascorbic acid are also electroactive. Therefore, the researchers attempted to minimize errors by interfering electroactive species in glucose sensors. In this case, the consumption of the oxygen by a mediator can be used to design more sophisticated glucose sensors.

Viologen derivatives have been widely investigated for their redox activity and electrochemical properties [3,4]. 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. A quartz crystal microbalance (QCM) is a powerful technique for biosensor applications that can detect nanogram changes of target material onto the electrode surface [5,6].

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In this work, thiol modified viologen was used to design the glucose biosensor as a redox mediator. The viologen derivative (VC10SH) played an important role as electron relays in systems in which electron transfer was initiated by electrochemical process [7]. It exhibited fast reversible electrochemical characteristics at negative potentials that can make it useful as the redox mediator for an enzymatic reaction [8]. A glucose oxidase (GOD) was immobilized onto a self-assembled viologen monolayer modified gold electrode. It is expected to increase the efficiency of the electron transfer and the sensitivity of the GOD modified electrode by viologen as the electron transfer mediator. The sensor based on this showed excellent performance, fast response, good sensitivity and low detection limit.

2. Experimental

2.1 Reagents

We synthesized the viologen derivative ($VC_{10}SH$) with Dong-Jin Qian (Fudan University, China). Fig. 1 shows a molecular structure of the viologen bonded with a thiol group. The glucose oxidase and glucose were purchased from Sigma. All other reagents were an analytical grade and used without any purification in this experiment. The stock solutions were prepared fresh with distilled water which was purified with a Milli-Q purification system.

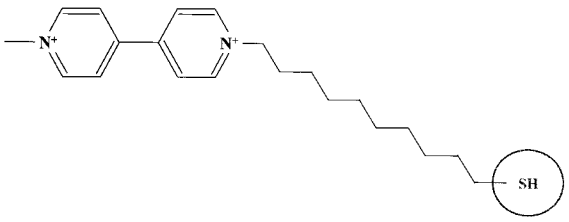

Fig. 1. The molecular structure of the viologen derivate

2.2 Electrode modification

At first, the gold electrode was cleaned by piranha solution (H_2SO_4:H_2O_2=3:1) and 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 100 mV/s scan rate until stable scans were recorded. The electrode was then thoroughly rinsed with water. After pretreatment, the electrode was immersed in an ethanol-acetonitrile (1:1) solution containing 2 mM thiol-functionalized viologen for 24 hours. After the self-assembly process, the electrode was removed from the deposition solution and rinsed with ethanol and water to remove weakly adsorbed viologen. Lastly, the viologen modified electrode was immersed into phosphate buffer saline (PBS) containing 5 mg/ml glucose oxidase for 5 hours. Fig. 2 shows the schematic process of the electrode modification.

2.3 Apparatus and electrochemical measurement

Fig. 3 shows the experimental system for measuring electrochemical and physical data, simultaneously. The cyclic voltammetry (CV) and chronoamperometry (CA) were carried out with the QCA 922 (Seiko EG&G, Japan) and potentiostat 263A (PerkinElmer, USA). The gold electrode, which was the self-assembled viologen monolayer with glucose oxidase onto the QCM, was used as a working electrode. A platinum wire and KCl saturated Ag/AgCl electrode were used as counter and reference electrodes, respectively. We observed the charge transfer property of the self-assembled viologen monolayer in PBS with pH 7.

3. Results and Discussion

The modified electrodes were characterized by cyclic voltammetry to confirm the electron flow from the gold electrode. Fig. 4 (a) shows the cyclic voltammogram of the GOD/Viologen/Au electrode in PBS at pH 7. The oxygen and reduction peak can be observed at about -0.6

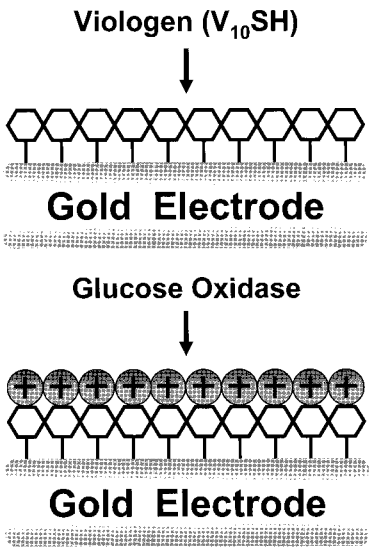

Fig. 2. The Schematic process of the electrode Modification

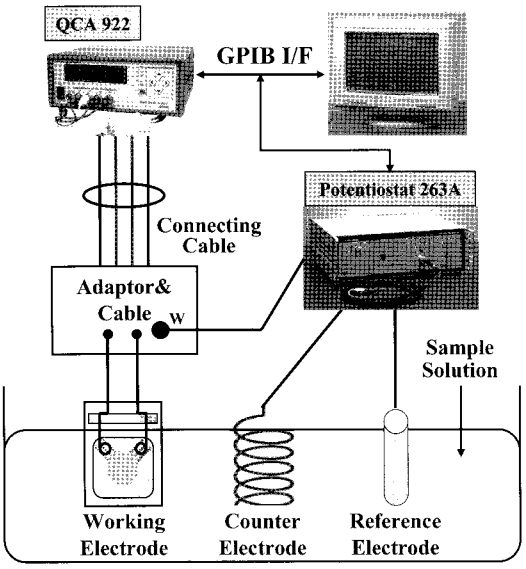

Fig. 3. The electrochemical 3-electrode setup using QCA and Potentiostat

V which is the same as typical redox peaks of the viologen. Fig. 4 (b) shows the cyclic voltammogram of the GOD/Viologen/ Au electrode in PBS containing 5 mM glucose. Interestingly, the reduction peak completely disappears in the presence of glucose. It is assumed that the catalytic effect of the oxygen reduction was caused by the viologen derivative. The mechanism of the oxygen reduction by viologen can be described as similarly as follows [9,10].

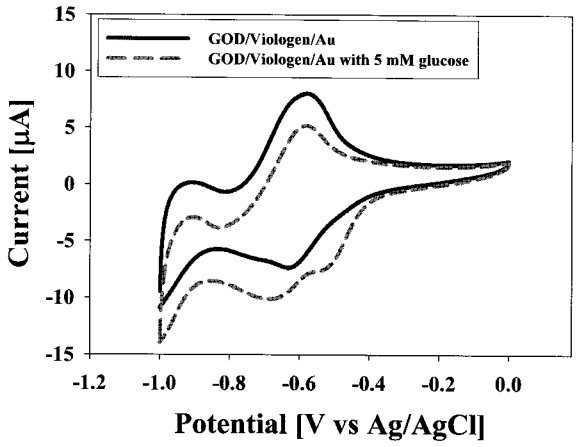

Fig. 4. The cyclic Voltammograms of GOD/Viologen/Au electrode in the absence and presence of 5 mM glucose

$V^{2+} + e^- \rightarrow V^{\bullet+}$

$V^{\bullet+} + O_2 \rightarrow V^{2+} + O_2^-$

$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$

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

Glucose + O_2 + H_2O → Gluconic acid +H_2O_2

The effect of the pH on the biosensor response is one of the most important factors. From this point of view, the pH effect was investigated in the presence of 5 × 10^{-5} M glucose in 0.1 M PBS. The current response of the biosensor was evaluated from pH 4 to pH 10. Fig. 5 shows the biosensor response for the glucose in various pH ranges. It can be seen that the current response increased from pH 4 to 7 and attained the maximum current at pH 7.

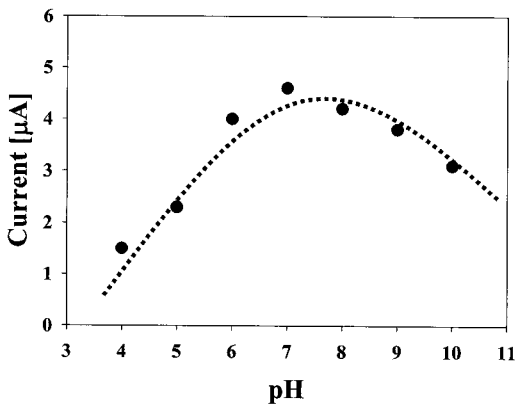

Fig. 5. The pH effect containing the solution of 5 × 10^{-5} M glucose in 0.1 M PBS at modified Electrode

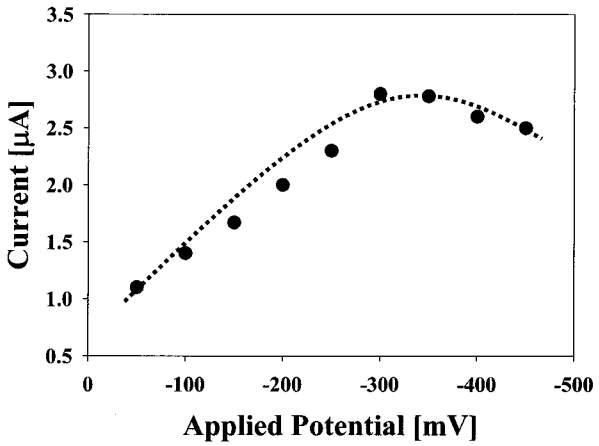

Fig. 6. The influence of the applied potential on amperometric response of the sensor

In strong basic or strong acidic solution, the biocompounds were denatured. Therefore, PBS with pH 7 was chosen as a supporting electrolyte in order to get maximum sensitivity and good bioactivity.

Fig. 6 shows the current response of the biosensor in various applied potential. The influence of the applied potential was studied in the presence of 5 × 10^{-5} M glucose. Based on the experimental data, the steady state current increases gradually as voltage increments from 0 mV to -300 mV. According to this experiment, the maximum current was achieved in the range of -300 mV. At more negative potential, there may be some risk of interfering reaction of the other electro-active species in the solution. Therefore, we have chosen applied potential -300 mV as the working potential for this sensor [11].

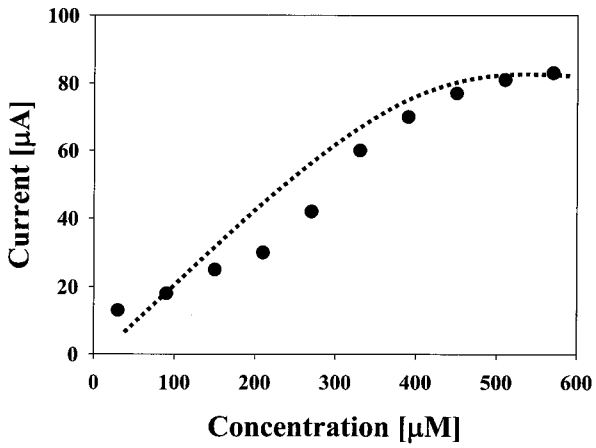

Fig. 7. The calibration plot of the modified electrode upon successive additions of the glucose

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 GOD/Viologen modified electrode to the glucose. It retained 80% of its initial current response after 30 days.

Fig. 7 shows the calibration plot of the GOD/Viologen/Au electrode under optimal conditions where the potential was kept at -300 mV in 0.1 M PBS with pH 7. It is confirmed that a well defined response was observed after successive additions of 1×10^{-5} M glucose. In the case of each injection, a sharp increase of the current response was observed less than 5 s. Through the optimal conditions, the plot of the current response shows a linear relationship in glucose concentration between 3.0×10^{-5} and 4.5×10^{-4} M with a detection limit of 3×10^{-6} M [12].

4. Conclusion

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

Acknowledgements

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