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Dual Glucose and Lactate Electrochemical Biosensor

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***Abstract*- We report a sensitive amperometric dual glucose and lactate biosensor based on pyroloquinoline quinone glucose dehydrogenase (PQQ-GDH) and a lactate dehydrogenase (LDH) for the detection of glucose and lactate, respectively. The electrochemical sensing platform comprise multi-walled carbon nanotubes (MWCNTs) for the immobilization of the PQQ-GDH and LDH at the respective working electrodes. Platinum and Ag/AgCl counter and reference electrodes were integrated to construct a three-electrode system. The lactate biosensor component exhibited a linear response towards lactate over a concentration range of 1 to 10 mM lactate ($r^2 = 0.998$) and exhibit a sensitivity of 2.9 µA mM^{-1} cm^{-2}, whereas the glucose biosensor exhibited a linear response range of 1 to 10 mM glucose ($r^2 = 0.990$) and a sensitivity of 345.7 µA mM^{-1} cm^{-2}. These results show that the dual glucose and lactate biosensor possess important potential for application in clinical analysis and sports medicine.**

I. Introduction

Glucose and lactic acid are essential metabolites present in body fluids and tissues. Lactic acid is a product of the anaerobic glycolysis of glucose and its level increases significantly in the case of hypoxia and/ or fatigue [1]. Therefore, the detection of glucose and lactate is important for clinical diagnosis and monitoring of organ or tissue viability during transplantation. Over one-hundred thousand people in the US are currently in need of a lifesaving organ/ tissue transplant [2]. In high risk situations, such as on the battlefield, organs retrieved in the line of duty will have to be transported to the appropriate hospital, thereby decreasing the time to preserve the organ. To prolong the lifespan of an organ, especially in such conditions, monitoring these two metabolic analyte while simultaneously treating and providing nutrients to the organ may improve organ preservation processes.

No commercialized organ preservation chambers, such as the TransMedic Organ Care System [3], are capable of monitoring blood metabolite. Also, this commonly used system is bulky and inconvenient for use in a high-risk situation. Currently developed biosensors for dual lactate and glucose sensing are also not suitable for organ preservation. For example, Yamazaki et al. developed an amperometric biosensor [4] that can be easily configured to monitor glucose and lactate concentrations; however, these biosensors exhibit inadequate performance when operating at physiological conditions due to the enzymes employed. Therefore, biosensors should be capable of operating in physiological conditions (37 □ and pH 7.4) and be sensitive to transient changes in glucose and lactate.

Amperometric biosensors can monitor blood metabolite levels [5-7] while offering a sensitive and selective means to monitor the analytes of interest [8]. Additionally, carbon nanotube (CNT) based electrochemical transducers offer substantial improvements in the performance of enzyme based electrodes due to their electrochemical reactivity [2]. The addition of CNT structures to enzymatic electrodes enhances the device sensitivity and the electroactive surface area. Furthermore, the CNTs provide very low cytotoxicity. In this work, we developed a dual glucose and lactate amperometric biosensor capable of sensing glucose and lactate with high sensitivity.

II. Materials and Methods

A. Electrode construction

4 mm × 4 mm buckypaper square electrodes were used for the preparation of the working electrodes. A 200 µm tungsten wire is affixed by a 4 mm × 1 mm strip of buckypaper and polyimide. Subsequently, polyimide was applied around three of the side edges to enhance the structural integrity of the buckypaper. The electrodes were cured at 150 °C for one hour. Upon curing, the bioelectrodes were flipped and polyimide was then applied on the backside and side edges of the bioelectrodes and cured again at 150 °C for one hour. The samples were stored under dry conditions and at room temperature until electrode surface modification and functionalization.

B. Electrode Surface Modification and Functionalization

Electrodes were rinsed in isopropyl alcohol for fifteen minutes to remove unbound particles. Ester moieties were introduced onto the surface of the buckypaper electrodes via π-π stacking of pyrenebutanoic acid, succinimidyl ester (PBSE) to enable the immobilization of PQQ-GDH and LDH on the buckypaper electrodes to realize the glucose and lactate biosensor components. Prior to enzyme immobilization, excess PBSE was rinsed with dimethyl sulfoxide for five minutes, then with phosphate buffer solution (PBS) to remove any unreacted PBSE from the electrode surface. The electroactive regions were coated with 2 µL of Nafion and dried in the desiccator at room temperature.

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The biosensors were stored at 4 °C in 100 mM PBS (pH 7.0) such that the electroactive regions were fully submerged. All electrochemical characterization were performed at 37 °C in 100 mM PBS (pH 7.4) unless otherwise noted. Cyclic voltammetry and chronoamperometry were performed in a glass electrochemical cell using three-electrode configuration with a platinum wire counter electrode versus a Ag/AgCl reference electrode (Fig. 1). A BASi Epsilon potentiostat was used. Cyclic voltammagrams (CVs) were carried out from an initial potential of -800 mV to a switching potential of 800 mV at a scan rate of 25 mV/s.

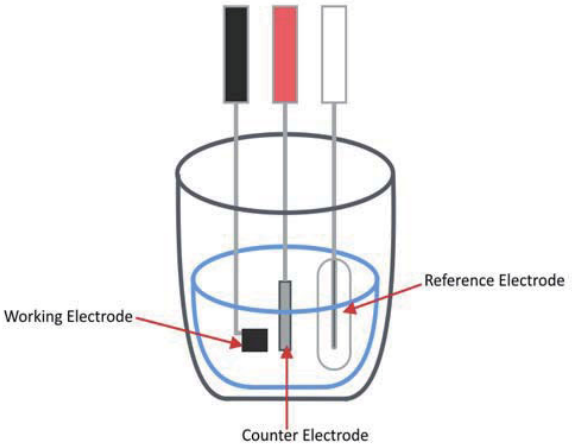

Fig. 1. Electrochemical set-up via three-electrode configuration. Temperature maintained at 37 °C.

III. Results

PQQ-GDH and LDH are immobilized on their respective electrode surface through a heterobifunctional crosslinking agent, PBSE, which created a shorter tethering distance between the enzyme and the solid buckypaper electrode support and minimized the hydrophobic interaction between

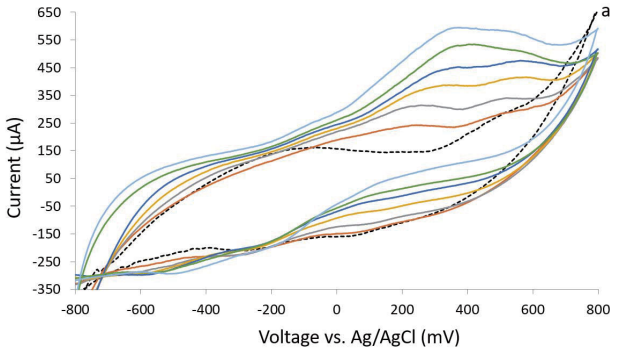

Fig. 2. Cyclic voltammograms for a glucose sensor in 1, 5, 10, 20 and 25 mM glucose (versus Ag/AgCl). (a) in the absence of glucose analyte.

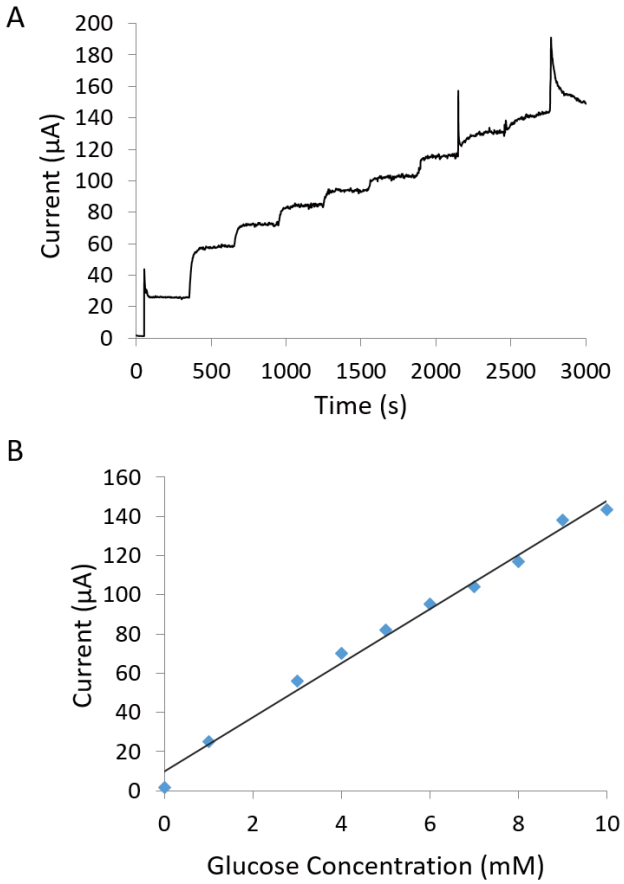

Fig. 3. A) Chronoamperometric response of the glucose biosensor with a successive addition of glucose solution in 0.1 M PBS solution pH 7.4 at working potential of 360 mV. B) Calibration plot of the response of glucose biosensor at pH 7.4 and 37 °C.

the enzyme and the multi-walled carbon nanotubes (MWCNTs) [9-11]. PBSE has a pyrene group that interacts with the MWCNT surface on buckypaper via π-π stacking. The PBSE ester group allows it to interact with primary amines on the surface of the enzyme, thereby forming a stable amide bond. This immobilization strategy has been shown to improve enzyme activity due to its confinement on a solid support [12-13]. The electrochemical behavior of the enzyme immobilized electrodes was assessed. MWCNTs have oxygen-containing defects such as quinone-type functional groups and metal impurities that possess electrochemical activity. Thereby, CV of bare buckypaper resulted in no redox peaks. The immobilized PQQ-GDH electrode produced redox peaks indicating redox potential. Fig. 2 shows the CVs of the PQQ-GDH working electrode in phosphate buffer solution as well as in various concentration of glucose [1, 5, 10, 20 and 25 mM]. The CVs show that there is a significant increase in current from PBS to glucose with a formal potential of -196 mV indicating that the biochemical recognition was catalyzed by

PQQ-GDH and the signal output was generated by transduction of the biorecognition event to current via the following electrochemical redox reaction:

$$Glucose \rightarrow Gluconolactone + 2\,H^{+} + 2\,e^{-} \quad (1)$$

The PQQ-GDH electrode in the absence of glucose (dashed black curve (a)) shows no observable response, whereas in the presence of glucose, the functionalized electrode exhibits obvious oxidation and reduction peaks. The peak current observed in the presence of glucose may be attributed to the intimate contact between the enzyme and the MWCNTs after PBSE crosslinking. During the forward potential scan, oxidation occurs and the peak oxidation can be observed at ca. 360 mV. These results revealed that glucose oxidation peaks at approximately 360 mV, therefore, this potential was applied during chronoamperometric characterization. Fig. 3A shows the chronoamperometric response 1 to 10 mM glucose. Furthermore, as shown in Fig. 3B, the calibration curve for the glucose biosensor demonstrates that the sensor exhibits a linear range of 1 to 10 mM glucose with a very high sensitivity of 345.7 µA $mM^{-1}cm^{-2}$. These results reflect the highly sensitive recognition of this MWCNT-based sensor.

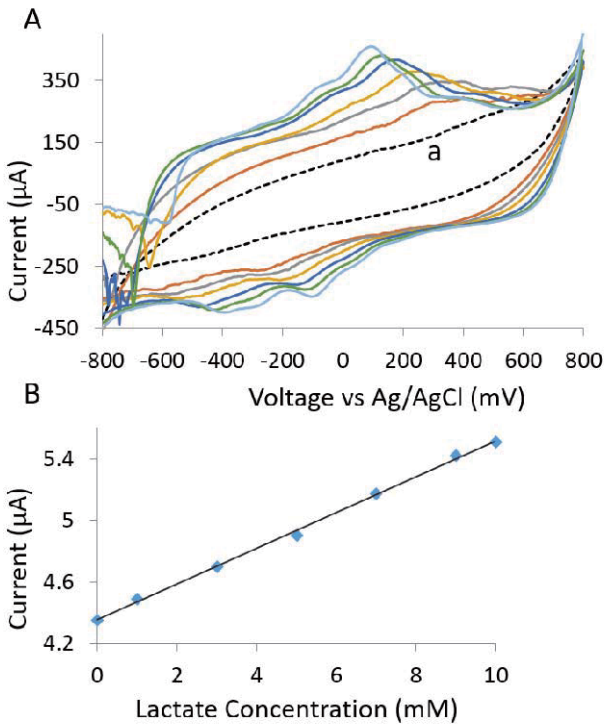

Fig. 4. Cyclic voltammograms for a lactate sensor in 1, 5, 10, 20 and 25 mM lactate (versus Ag/AgCl). (a) in the absence of lactate analyte.

Fig. 4A depicts the CVs for the LDH working electrode in the absence and presence of lactic acid solution. The biochemical recognition event in this case was catalyzed by LDH and the signal output was generated by transduction of the biorecognition event to current via the following electrochemical redox reaction:

$$Lactic\ acid \rightarrow Pyruvate + 2\,H^{+} + 2\,e^{-} \quad (2)$$

Similar to the glucose electrode, the LDH electrode exhibits no observable response in the absence of lactate (dashed black curve (a)), whereas in the presence of lactate an observable peak current for the oxidation of lactic acid was prominent. During the forward potential scan, lactate oxidation occurs at an onset potential of ca. -178 mV. The dynamic sensor response as function of lactate concentration was subsequently investigated via chronoamperometric. Fig. 4B shows the calibration curve obtained in 1 mM to 10 mM lactate. The lactate sensor displays a good lactate concentration dependence, with a logistic increasing curve response from 1 mM to 10 mM and a sensitive of 2.9 µA mM^{-1} cm^{-2}. Importantly, this dual glucose and lactate biosensor displays a higher sensitivity, which may be attributed to the large specific surface area of the MWCNTs and the tethering of enzyme via PBSE to enable enzyme immobilization and charge conduction.

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