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An electrochemically preanodized screen-printed carbon electrode for achieving direct electron transfer to glucose oxidase

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ABSTRACT

Here we report the unique property of a preanodized screen-printed carbon electrode (SPCE*) that can allow direct electron transfer (DET) reaction of glucose oxidase (GOx). The GOx can be immobilized in the composite of oxygen functionalities and edge plane sites generated during preanodization without additional cross-linking agents. The electron transfer rate of GOx is greatly enhanced to 4.38 s^{-1} as a result of the conformational change of GOx in the microenvironment enabling the accessibility of active site for GOx to the electrode. The analytical versatility is further improved with the aid of Nafion film. As a consequence, the as-prepared electrode can be used as a glucose biosensor and the number of potential foreign species is then restricted by molecular size, permeation and/or (bio)chemical reaction. Most importantly, the disposable nature of the proposed electrode is expected to promote the DET-related researches.

1. Introduction

The direct electron transfer (DET) from the redox center (i.e., flavin adenine dinucleotide, FAD/$FADH_2$) of glucose oxidase (GOx) to electrode is difficult to observe because the active site is deeply embedded within a protective protein shell. To improve the communication between active site and electrode, one successful approach generally referred to as "wired" enzyme electrodes has involved binding redox-active centers (mediators) and enzymes in a polymeric matrix immobilized on an electrode surface [1–6]. Nonetheless, electrode interfaces need to be designed containing a pathway that allows efficient electron transfer and the complicated procedure usually limits their broad application. The use of a suitable matrix to immobilize GOx is a more convenient way to accomplish the purpose of DET and even with higher sensitivity and better stability to act as glucose biosensors [7–15]. It was reported that carbon nanotube (CNT) can promote the DET due to the presence of the oxygen-contained groups on the surface [13,16,17]. The effect of oxygenated species at CNT was studied and the rate of electron transfer was found to be dominated by the functionalities in the CNT end, especially the carboxylate moieties [18,19]. On the other hand, Compton's group studied the location and nature of electron transfer processes on CNT-modified electrodes and concluded that the edge plane-like defects on CNT as the key to the high electrocatalytic activity toward several biological molecules [20–24].

In our earlier studies we have reported exclusive applications of disposable preanodized screen-printed carbon electrode (designated as SPCE*) with improved electrochemical activity [25–29]. The disposable SPCE* with the introduction of edge plane carbonyl groups was found to act more or less like an edge plane graphite electrode or CNT. Based on flexibility and robustness of the SPCE*, a simple and facile alternate to allow DET reaction of GOx and the discussion on enzyme denaturation are established in this study. Note that Dong and Wang previously reported that DET reaction can take place between the adsorbed GOx and the anodized glassy carbon electrode, but the adsorbed enzyme can not catalyze its substrate due to its denaturation [30]. They suggest that the adsorbed enzyme loses its native bioactivity owing to the great change in the structure, i.e., the GOx molecule adsorbs onto the anodized electrode surface in a holoenzyme form and then extends gradually to an unfolded structure. This is, however, not the case at the SPCE* as the GOx can retain its bioactivity. Overall, the fact that GOx can be immobilized easily without additional cross-linking agents together with the disposable nature of the proposed electrode is expected to promote the DET-related researches.

2. Experimental

GOx (EC 1.1.3.4 from *Aspergillus niger*, Sigma), Nafion (5 wt% in mixture of lower aliphatic alcohols and water, Aldrich), β-D-glucose, Na_2HPO_4, NaH_2PO_4, uric acid and ascorbic acid were of ACS certified reagent grade. A pH 7.4 phosphate buffer solution (PBS) was used in all studies. Water was obtained from a Millipore purification system. Voltammetric measurements were carried out

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with a CH Instruments (CHI 627) electrochemical workstation in a three-electrode cell assembly. A bare SPCE or SPCE* (working electrode), an Ag/AgCl, 3 M KCl (reference electrode) and a platinum wire (auxiliary electrode) were used to complete the cell setup. The SPCE with a working area of 0.196 cm^2 and a conductive track radius of 2.5 mm was purchased from Zensor R&D (Taichung, Taiwan). Electrodes were then preanodized by applying a potential at 2.0 V vs. Ag/AgCl for 300 s in pH 7.4 PBS. The GOx solution was prepared by dissolving 20 mg GOx in 1 mL PBS.

3. Results and discussion

Fig. 1 compares the cyclic voltammograms at SPCEs with/without preanodization and enzyme-immobilization in pH 7.4 PBS. For enzyme-immobilization, the GOx solution (5 μL) was spread evenly onto the working electrode surface with a microsyringe and let dried for 2 h. As can be seen, no peak responses were observed at SPCEs without enzyme-immobilization (curves a and c). A pair of well-defined and nearly symmetric redox peaks can only be observed with a formal potential of −0.45 V at the SPCE* with enzyme-immobilization (curve d). The controlled experiments clearly indicate that the redox peaks are derived from GOx, which is similar to the reported results [7,13,15]. The electrochemical response of GOx immobilized onto the heterogeneous surface is due to a redox reaction of FAD/$FADH_2$ [10]. Note that FAD is known to undergo a two-electron coupled with two-proton redox reaction, i.e., GOx/FAD + $2e^-$ + $2H^+$ = GOx/$FADH_2$. Thus the anodic and cathodic peak potentials of GOx immobilized on the surface of SPCE* should be pH-dependent. This is indeed the case for our system. As shown in Fig. 2, an increase of the solution pH leads to a negative shift in potential for both anodic and cathodic peaks. The slope for a linear plot of $E_{1/2}$ vs. pH is –62 mV/pH, which is close to the theoretical one (–59 mV/pH) for a reversible, two-proton coupled with two-electron redox reaction process. Overall, the oxygen functionalities and edge plane-like sites formed at the SPCE* play an important role in facilitating the electron transfer between the GOx and electrode.

Fig. 3A shows the cyclic voltammograms at different scan rates ranged from 10 to 150 mV/s. A good linear relationship for the peak current and scan rate (Fig. 3B) indicates a surface-controlled electrode process. An estimation of the electron transfer rate constant (k_s) can be made from the peak-to-peak separation value using the model of Laviron [26,31,32]. Taking a charge transfer coefficient α

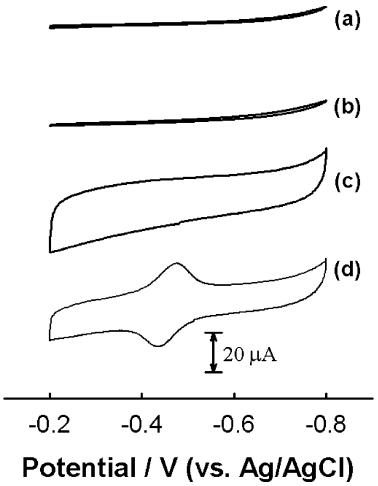

Fig. 1. Cyclic voltammetric responses of a bare SPCE (a), SPCE immobilized with GOx (b), SPCE* (c) and SPCE* immobilized with GOx (d) in 0.1 M, pH 7.4 PBS. Scan rate = 50 mV/s.

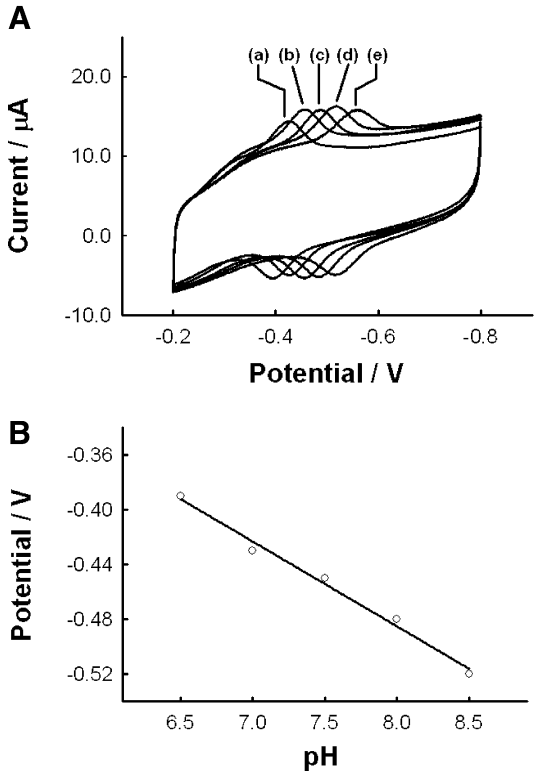

Fig. 2. Cyclic voltammetric responses of the SPCE* immobilized with GOx in different pH (a–e: 6.5, 7.0, 7.5, 8.0 and 8.5) phosphate solutions. Inset shows the plot of E_p vs. pH. Scan rate = 50 mV/s.

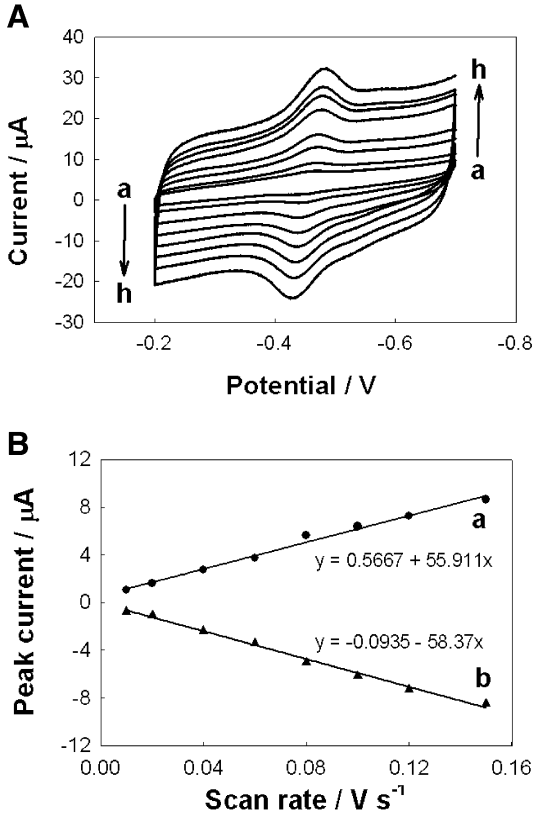

Fig. 3. (A) Cyclic voltammetric responses of the SPCE* immobilized with GOx in 0.1 M, pH 7.4 PBS with different scan rates (a–h: 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.15 V/s). (B) Plots of cathodic (a) and anodic (b) peak current with scan rate.