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# Study of direct electron transfer and enzyme activity of glucose oxidase on graphene surface

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ABSTRACT

In recent years, graphene has been widely used as a high performance two-dimensional material in the development of biosensors and biofuel cells for facilitating direct electron transfer (DET) of glucose oxidase (GOx). However, almost all of these reports perform experiments in the presence of oxygen (a natural mediator of oxidase) and whether the GOx with DET property retained their catalytic activity in the absence of mediators has not been studied in detail so far. In this paper, we investigated the DET property and enzyme activity of GOx on graphene surface without and with mediators. Experimental results showed that the biosensor had no response to glucose in mediator-free solutions, even though the DET of GOx was observed, indicating that the GOx with DET property lacked enzymatically catalytic activity. However, in the presence of mediators, the biosensor showed sensitive response to glucose, illustrating that the mediated enzymatic oxidation of glucose occurred, which can be attributed to the catalytically active GOx without DET capability. These results suggest that DET property and enzyme catalytic activity cannot occur on the same GOx simultaneously. Therefore, keeping enzyme activity and DET of GOx at the same time is still a major challenge for biosensor and biofuel cell researches.



## 1. Introduction

The direct electron transfer (DET) between enzymatically active glucose oxidase (GOx) and conductive electrode surface is crucial for the development of mediator-less biosensors [1–4] and high performance biofuel cells [5–7]. The DET-based enzymatic biosensor is an ideal detection technique, possessing several advantages such as reagent-less sensing, low operation potential, and high selectivity [8–10]. The DET-based enzymatic biofuel cells can work in a potential range close to the redox potential of the enzyme itself and give the maximum potential difference between the anode and cathode, resulting in a higher power output [11–13].

The DET of enzymatically active GOx is extremely difficult, since the active site of GOx, flavin adenine dinucleotide (FAD), is deeply embedded within a protective protein shell [14,15]. Thus, various nanomaterial, such as gold nanoparticles [16,17], carbon nanotubes [18,19], graphene [20,21] and their composites [22–24] have been used to promote the electron transfer of redox proteins. However, recent studies of DET and enzyme activity of GOx on carbon nanotubes showed that DET did not occur between enzymatically active GOx and carbon nanotubes [25–27].

Graphene has attracted considerable attention from both the experimental and theoretical scientific communities in recent years [28,29]. Owing to the excellent electrical, chemical and mechanical properties, graphene has stimulated exploding interest in sensor applications [30–32]. Numerous studies of DET behavior of GOx on graphene decorated electrodes have been reported [33–38]. In our previous research, we also studied the DET of GOx self-assembled on graphene surface [39]. However, almost all of these reports examined glucose detection performances in the presence of oxygen, indicating that those glucose biosensors were not the real mediator-less DET-based biosensors. In fact, the real meaning of DET is between enzymatically active GOx and electrode. Up to now, whether the GOx on graphene surface with DET performance retained their enzyme activity and whether the graphene based biosensor can be used for glucose detection without mediators have not been studied detailedly.

Here, we investigated the DET property and enzyme activity of GOx on graphene modified electrode in mediator-free and mediator containing solutions. Experimental results show that GOx with DET property lost their enzyme activity when adsorbed onto graphene surface while GOx with enzyme activity couldn't direct transfer electrons to electrode.

## 2. Experimental

Graphene modified electrode was prepared by electrochemical reduction of graphene oxide (GO) as previously reported [39–41].

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Briefly, 6 μL GO water dispersion (1 mg/mL) was cast on the polished glassy carbon electrode (GCE) surface and dried in the ambient condition. 5 cycles of cyclic voltammetric scanning from 0.7 to −0.9 V at a scan rate of 50 mV $s^{-1}$ in $N_2$-saturated 0.5 M NaCl solution were performed. The electrode was denoted as ERGO/GCE. Then 6 μL GOx/PBS solution (10 mg/mL GOx, pH 7.4) was cast on the ERGO/GCE surface, dried in the ambient condition. Afterwards, 6 μL 1% Nafion was cast on the electrode surface to maintain the stability of the modified electrode. The final electrode denoted as GOx/ERGO/GCE was rinsed thoroughly with deionized water and dried in air. For comparison, bare GCE, GOx/GCE and ERGO/GCE were prepared respectively, and were casted with 6 μL 1% Nafion finally.

## 3. Results and discussion

### 3.1. DET between GOx and electrode in $N_2$-saturated solution

DET of GOx adsorbed on ERGO modified GCE was studied by cyclic voltammetry in $N_2$-saturated PBS. Fig. 1A shows the cyclic voltammograms (CVs) of GCE with or without ERGO and GOx modification. A pair of well-defined and quasi-reversible redox peaks was observed at the CVs of GOx/ERGO/GCE (Fig. 1A line 4). The formal potential of the redox peaks was −0.48 V, and the separation of peak potentials was 40 mV, which is close to redox peaks of GOx and its cofactor FAD on modified electrode surfaces in previous reports [20,42]. However, no redox peaks were observed at the CVs of bare GCE (Fig. 1A line 1) and ERGO/GCE (Fig. 1A line 3), indicating that the redox peaks of the GOx/ERGO/GCE should be ascribed only to GOx. What's more, when GOx was directly adsorbed onto a bare GCE surface, no redox peaks were observed (Fig. 1A line 2), suggesting that ERGO modification on GCE is necessary to facilitate the DET of GOx. The observed redox is due to the electron transfer of the cofactor FAD of GOx via reaction (1):

$$GOx(FAD) + 2H^+ + 2e^- \leftrightarrow GOx(FADH_2) \quad (1)$$

According to reaction (1) the DET of GOx is a two-electron and two-proton coupled reaction. The cathodic peak current is attributed to the reduction of GOx(FAD), while the anodic peak current is attributed to the oxidation of $GOx(FADH_2)$. The pH value of the solution will influence the direct electron transfer of GOx. As shown in Fig. 1B, both the anodic and cathodic peak potentials shift to negative direction with the increase of the solution pH. The formal potential exhibits a linear dependence on the pH ranging from 5 to 9 with a slope of −59 mV/pH (r = 0.999).

As we know, the DET-based third generation glucose biosensor doesn't need any mediators, including oxygen. Glucose is oxidized by GOx into gluconolactone while GOx(FAD) is reduced into $GOx(FADH_2)$, and then the GOx is directly recycled at the electrode surface by electrochemical oxidation of $GOx(FADH_2)$. Both reactions are shown as follows:

$$GOx(FAD) + glucose \rightarrow GOx(FADH_2) + gluconolactone \quad (2)$$

$$GOx(FAD_2) \rightarrow GOx(FAD) + 2H^+ + 2e^- \quad (3)$$

However, when glucose was added into the solution, the CVs of GOx/ERGO/GCE had no variation (Fig. 1C). The lack of glucose response indicated that no enzymatic reaction happened to GOx with DET capability; otherwise GOx(FAD) could be reduced into $GOx(FADH_2)$

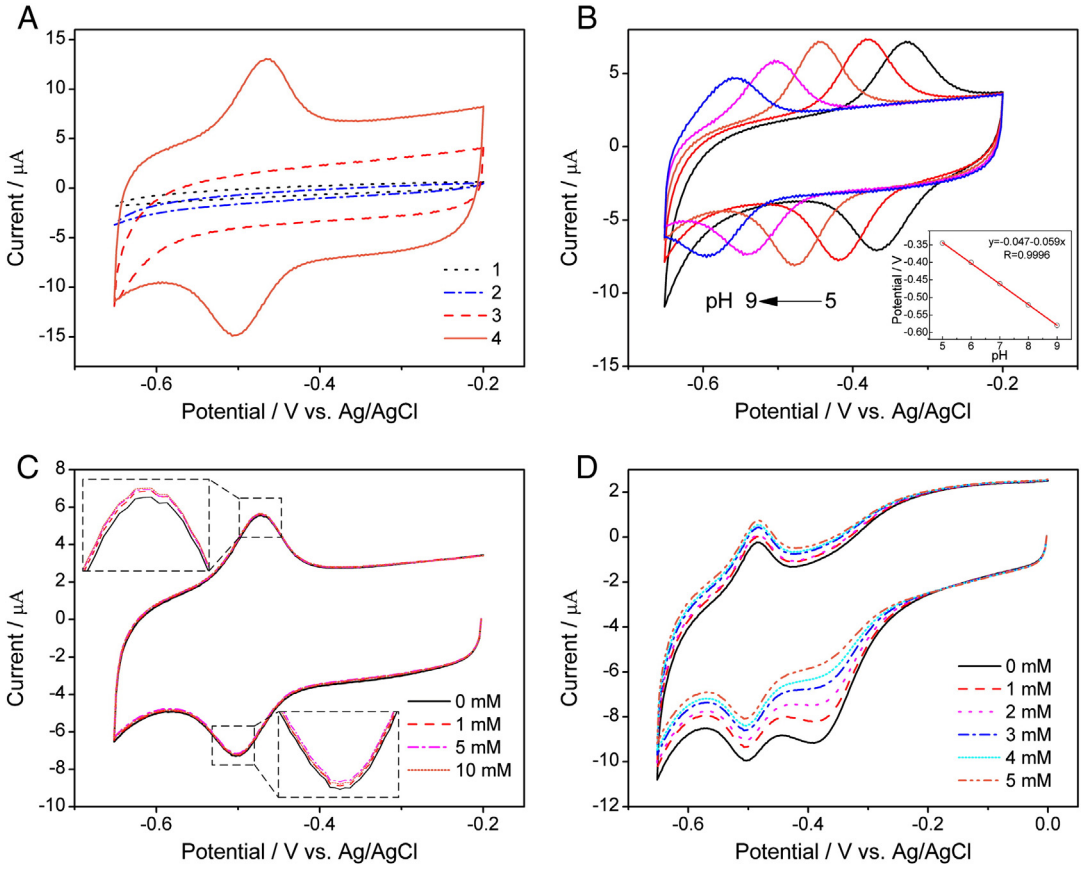


**Fig. 1.** (A) CVs of bare GCE (1), GOx/GCE (2) ERGO/GCE (3), and GOx/ERGO/GCE (4) in $N_2$-saturated PBS. (B) CVs of GOx/ERGO/GCE in $N_2$-saturated PBS with different pH values of 5, 6, 7, 8 and 9. The inset is the plot of formal potential vs. pH. (C) CVs of GOx/ERGO/GCE in $N_2$-saturated PBS with 0 mM, 1 mM, 5 mM, and 10 mM glucose respectively. Insets: enlargement of the redox peak areas (dashed box). (D) CVs of GOx/ERGO/GCE in air-saturated PBS in various concentrations of glucose. Scan rates, panel A: 50 mV $s^{-1}$, panel B, C, and D: 25 mV $s^{-1}$.