Reagentless Glucose Biosensor Based on the Direct Electrochemistry of Glucose Oxidase on Carbon Nanotube-Modified Electrodes

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Abstract
The direct electrochemistry of glucose oxidase (GOD) was revealed at a carbon nanotube (CNT)-modified glassy carbon electrode, where the enzyme was immobilized with a chitosan film containing gold nanoparticles. The immobilized GOD displays a pair of redox peaks in pH 7.4 phosphate buffer solutions (PBS) with the formal potential of about − 455 mV (vs. Ag/AgCl) and shows a surface-controlled electrode process. Bioactivity remains good, along with effective catalysis of the reduction of oxygen. In the presence of dissolved oxygen, the reduction peak current decreased gradually with the addition of glucose, which could be used for reagentless detection of glucose with a linear range from 0.04 to 1.0 mM. The proposed glucose biosensor exhibited high sensitivity, good stability and reproducibility, and was also insensitive to common interferences such as ascorbic and uric acid. The excellent performance of the reagentless biosensor is attributed to the effective enhancement of electron transfer between enzyme and electrode surface by CNTs, and the biocompatible environment that the chitosan film containing gold nanoparticles provides for immobilized GOD.

Keywords: Glucose oxidase, Carbon nanotubes, Biosensor, Direct electrochemistry, Reagentless

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The direct electron transfer (DET) between redox enzymes and electrode surfaces has been extensively studied in recent years, and it is still an important research subject due to its application in the areas of biochemical and biophysical sciences [1, 2]. Unfortunately, redox enzymes usually have difficulty in displaying DET at conventional electrode surfaces due to their three-dimensional structure and the shielding of redox active centers by their insulated protein shells. Many strategies have been proposed to promote the DET of redox enzymes with electrode surfaces, and one of the promising ways is the application of nanomaterials [3, 4].

Glucose oxidase (GOD) is a type of glycoprotein containing two flavin adenine dinucleotide (FAD) cofactors, which are deeply embedded in the protein shell [5]. The DET of GOD, although inherently difficult, has been realized at electrodes modified with nanomaterials, such as gold nanoparticles [6] and carbon nanotubes (CNTs) [7 – 12]. Although some studies have demonstrated DET of GOD, these platforms cannot be further developed due to the loss of activity of the immobilized GOD [10, 13]. Among the researchers who have overcome this problem are Cai et al. [11] and Dong et al. [12]. Both groups have successfully obtained the DET of GOD with CNT-modified electrodes, where the immobilized GOD was found to be still active. However, the development of glucose biosensors based on their systems required extra mediators. Ju et al. [6] developed a reagentless biosensor based on carbon paste electrodes modified with gold nanoparticles, but the linear range of their biosensor toward glucose was rather narrow (0.04 – 0.28 mM). Luong and co-workers [9] also developed reagentless biosensors through the immobilization of GOD onto CNT-modified electrodes, but their sensor preparation was complicated, and the response mechanism of their biosensor was not clear. Therefore, as to the attempt of achieving reagentless glucose biosensors based on the DET of GOD with the application of nanomaterials, the results are far from satisfying. Here, we investigated the DET of GOD on CNT-modified glassy carbon electrodes, and successfully developed a reagentless glucose biosensor.

The cyclic voltammograms of different modified electrodes were studied in deaerated PBS, and a couple of redox peaks with a formal potential of − 0.455 V (vs. Ag/AgCl) were observed at the GOD/CNT-modified electrode, as shown in Figure 1. In contrast, no peak was observed at both bare and CNT-modified glassy carbon electrodes, and the presence of CNTs resulted in an obvious increase in the background current due to the increased active electrode area. Meanwhile, there was no peak at the electrode modified with GOD alone. Therefore, the redox peaks observed at the GOD/CNT-modified electrode were attributed to the direct electrochemistry of GOD, and CNTs play a key role in facilitating the electron transfer directly between GOD and the electrode surface.

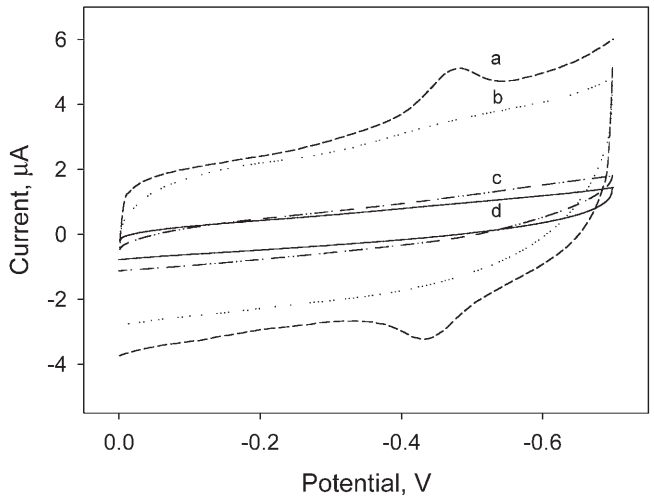

Fig. 1. Cyclic voltammograms of GOD/CNT-modified (a), CNT-modified (b), GOD-modified (c), and bare (d) glassy carbon electrodes in pH 7.4 N_2-saturated PBS at a scan rate of 50 mV/s.

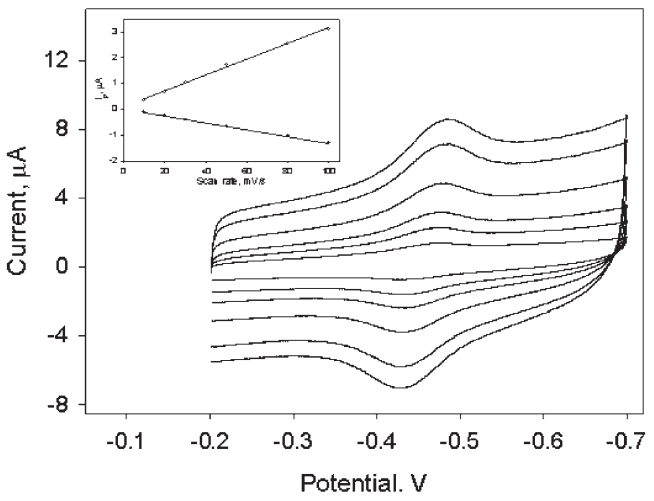

Fig. 2. Cyclic voltammograms of GOD/CNT-modified glassy carbon electrodes in pH 7.4 N_2-saturated PBS at a scan rates of 10, 20, 30, 50, 80 and 100 mV/s (from inner to outer). Inset: Plots of peak currents vs. scan rates.

The scan rate dependence of the redox peak currents and the peak-to-peak separation was studied, and the results are shown in Figure 2. The redox peak currents increased with increasing scan rate, and the anodic and cathodic peak currents for GOD were both linearly proportional to the scan rate ranging from 10 to 100 mV/s, indicating a surface-controlled electrochemical process. The peak-to-peak separation (ΔE_p) of GOD also increased slightly with the increase of scan rate, and based on the ΔE_p at different scan rates, the electron transfer rate constant, k_s, was estimated to be about 1.08 s^{-1} according to the model of Laviron [14].

As is known, the direct electrochemistry of GOD is based on the redox of its active center, FAD. In the absence of oxygen, the DET of immobilized GOD in our system can be expressed as the following:

$$GOD(FAD) + 2H^+ + 2e^- \rightleftharpoons GOD(FADH_2) \quad (1)$$

In the presence of oxygen – the natural substrate of GOD – the reduced form of GOD is quickly oxidized by oxygen according to the following reaction:

$$GOD(FADH_2) + O_2 \rightarrow GOD(FAD) + H_2O_2 \quad (2)$$

Therefore, the direct oxidation of $GOD(FADH_2)$ at the electrode surface is greatly suppressed, resulting in the decrease of the oxidation peak current; while at the same time, the rapidly produced GOD(FAD) through Reaction 2 will be reduced according to Reaction 1, leading to the obvious increase of the reduction peak current, as shown in Figure 3b. To confirm whether the immobilized GOD retains its enzymatic activity while demonstrating DET, glucose was added to the PBS in the presence of oxygen, as active GOD will catalyze the oxidation of glucose:

$$GOD(FAD) + Glucose \rightarrow GOD(FADH_2) + Gluconolactone \quad (3)$$

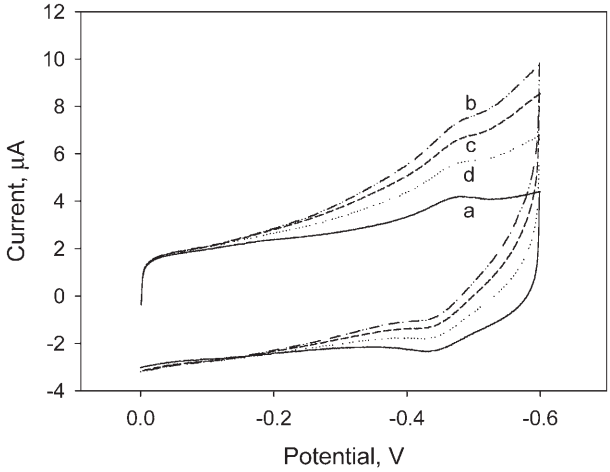

Fig. 3. Cyclic voltammograms of GOD/CNT-modified glassy carbon electrodes in pH 7.4 PBS in the absence (a) and presence of dissolved oxygen with 0 (b), 0.2 (c), and 0.6 (d) mM glucose. Scan rate: 50 mV/s. The reduction current is taken as positive.

In theory, the reduction of GOD(FAD) with glucose will cause the decrease of the reduction peak current resulting from the direct reduction of GOD(FAD) at the electrode surface. As can be seen from scans c and d in Figure 3, the reduction peak current of GOD decreased with the addition of glucose, which suggests that the immobilized GOD, while exhibiting direct electrochemistry, still retains its enzymatic activity and is sensitive to glucose. Therefore, a reagentless glucose biosensor can be developed based on the changes of reduction peak current resulting from the addition of glucose. Figure 4 shows the amperometric response of the glucose biosensor in the presence and absence of oxygen at -0.48 V (vs. Ag/AgCl). In the presence of oxygen, an obvious glucose response was observed, which was consistent with the results of the earlier cyclic voltammetry

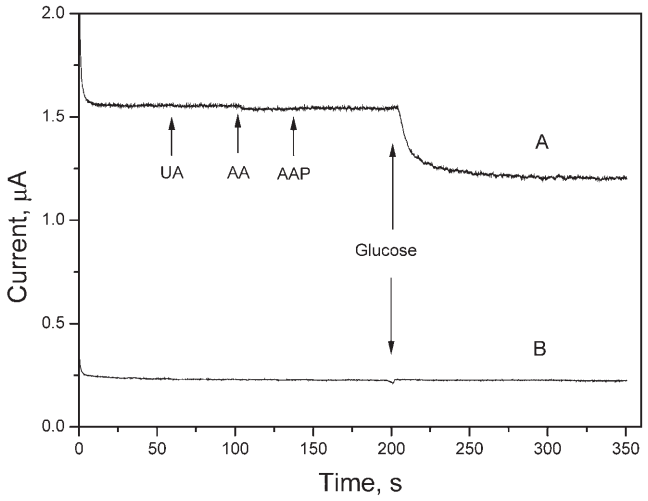

Fig. 4. Amperometric response of GOD/CNT-modified glassy carbon electrode toward the same concentration of glucose at −0.48 V (vs. Ag/AgCl) in pH 7.4 PBS in the presence (A) and absence (B) of oxygen. Curve A also shows the response on the additions of 0.1 mM uric acid (UA), ascorbic acid (AA) and acetaminophen (AAP).

experiments. However, in the absence of oxygen, the addition of glucose resulted in no response at all.

The performance of the proposed glucose biosensor was investigated. It exhibited a linear response to glucose in the concentration range from 0.04 to 1.0 mM ($n = 9$, $r = 0.999$), which was wider than the previously reported 0.04–0.28 mM [6]. The detection limit of the biosensor was estimated to be 0.02 mM at a signal-to-noise ratio of 3, and the sensitivity of the biosensor was about 2.40 μA/mM.

The biosensor was stored at 4 °C and measured at intervals over several days, and it retained about 87% of its original sensitivity after one month. The relative standard deviation (*RSD*) of the biosensor response to glucose was 3.4% for nine successive measurements. The fabrication reproducibility of three biosensors, made independently, showed reproducibility with the RSD of 2.7% for the detection of glucose. The possible interference from ascorbic acid, uric acid and acetaminophen was investigated, and it was found that the relevant physiological levels (approx. 0.1 mM) of these three common interfering species only resulted in negligible responses, as shown in Figure 4.

In conclusion, the direct electrochemistry of GOD was realized at a CNT-modified glassy carbon electrode through the immobilization of GOD with a gold nanoparticle-containing chitosan film. The immobilized GOD retained its enzymatic activity due to the gold nanoparticle/chitosan film, while exhibiting good DET due to the presence of the CNT-modified electrode surface. As a result, a mediatorless glucose biosensor was developed accordingly. The excellent performance of the proposed biosensor, such as good stability, high sensitivity and freedom from interference, makes it promising for glucose detection. Moreover, considering its simplicity in preparation, this technique could also be used to develop biofuel cells and other biomedical devices based on the direct electrochemistry of enzymes.

Experimental

GOD (EC 1.1.3.4), Chitosan (85% deacetylated) and Dimethyl formamide (DMF, 99.8%) were purchased from Sigma-Aldrich. Multiwalled CNTs (95%, 10–20 nm) were purchased from Shenzhen Nanotech. Port. Co. Ltd. (Shenzhen, China). All other chemicals were of analytical grade, and Milli-Q water (resistance over 18 MΩ cm) from a Millipore Q water purification system was used throughout. The preparation of gold nanoparticles and chitosan solution was according to the work of Luo et al. [15].

Electrochemical experiments were performed on a CHI 1000 electrochemical workstation (CH Instruments, USA) at room temperature in pH 7.4 phosphate buffer solution (PBS), using a conventional three-electrode system with the modified electrode as the working electrode, a platinum mesh as the auxiliary electrode, and a silver/silver chloride (Ag/AgCl) reference electrode.

CNTs were dispersed in DMF with ultrasonication (1.0 mg/mL) for 20 min, and 5 μL of the resulted CNT suspension was dropped onto the cleaned glassy carbon electrode ($\Phi = 3$ mm) surface with pipette. After the evaporation of DMF in air, the resulting CNT-modified electrode was used for enzyme immobilization according to Luo et al. [15]. Briefly, the CNT-modified electrode was immersed in 2.0 mL 0.5% (w/v) chitosan solution containing 0.8 nM gold nanoparticles (about 17 nm) and 5.0 mg/mL GOD, and a potential of −3.0 V was applied to the electrode to electrodeposit chitosan. With the electrodeposition of chitosan, gold nanoparticles and GOD were entrapped into the chitosan film.

Acknowledgements

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References

[1] S. D. Varfolomeev, I. N. Kurochkin, A. I. Yaropolov, *Biosens. Bioelectron.* **1996**, *11*, 863.
[2] S. C. Barton, J. Gallaway, P. Atanassov, *Chem. Rev.* **2004**, *104*, 4867.
[3] S. Q. Liu, D. Leech, H. X. Ju, *Anal. Lett.* **2003**, *36*, 1.
[4] M. K. Wang, Y. Shen, Y. Liu, T. Wang, F. Zhao, B. F. Liu, S. J. Dong, *J. Electroanal. Chem.* **2005**, *578*, 121.
[5] H. J. Hecht, H. M. Kalisz, J. Hendle, R. D. Schmid, D. Schomburg, *J. Mol. Biol.* **1993**, *229*, 153.
[6] S. Q. Liu, H. X. Ju, *Biosens. Bioelectron.* **2003**, *19*, 177.
[7] A. Guiseppi-Elie, C. H. Lei, R. H. Baughman, *Nanotechnology* **2002**, *13*, 559.
[8] J. Q. Liu, A. Chou, W. Rahmat, M. N. Paddon-Row, J. J. Gooding, *Electroanalysis* **2005**, *17*, 38.
[9] J. H. T. Luong, S. Hrapovic, D. Wang, F. Bensebaa, B. Simard, *Electroanalysis* **2004**, *16*, 132.

[10] Y. D. Zhao, W. D. Zhang, H. Chen, Q. M. Luo, *Anal. Sci.* **2002**, *18*, 939.
[11] C. X. Cai, J. Chen, *Anal. Biochem.* **2004**, *332*, 75.
[12] Y. Liu, M. K. Wang, F. Zhao, Z. A. Xu, S. J. Dong, *Biosens. Bioelectron.* **2005**, *21*, 984.
[13] Q. J. Chi, J. D. Zhang, S. J. Dong, E. K. Wang, *Electrochim. Acta* **1994**, *39*, 2431.
[14] E. Laviron, *J. Electroanal. Chem.* **1979**, *101*, 19.
[15] X. L. Luo, J. J. Xu, Y. Du, H. Y. Chen, *Anal. Biochem.* **2004**, *334*, 284.