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Direct electrochemistry of glucose oxidase and biosensing for glucose based on DNA/chitosan film

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Abstract

Glucose oxidase (GOD) is widely used in the glucose biosensor industry. The amperometric biosensors based on directly electron transfer (DET) between an electrode and immobilized GOD are especially promising. In this article, GOD was immobilized with a DNA/chitosan bio-material film on GC electrode, and the DET of GOD on DNA/chitosan was studied. The cyclic voltammetric results indicated that the GOD immobilized in the DNA/chitosan film underwent DET reaction, and the cyclic voltammogram displayed a pair of well-defined redox peaks with a formal potential of –0.45 V (vs. Ag/AgCl) at pH 5.5 . The response showed a surface-controlled electrode process with an electron transfer rate constant of 0.91 sec^{-1} determined in the scan rate range from 10 to 100 mV/sec. The GOD immobilized in DNA/chitosan membrane retained its biocatalytic activity and stability. The immobilized GOD could electrocatalyze the reduction of dissolved oxygen and resulted in a great increase of the reduction peak current. Upon the addition of glucose, the reduction peak current decreased, which could be used for glucose detection with a sensitivity of 0.48 μA/(mmol/L), a linear range from 0.04 to 2.28 mmol/L and a detection limit of 0.04 mmol/L at a signal-to-noise ratio of 3. The sensor could exclude the interference of commonly coexisted uricacid and ascorbic acid.

Key words: glucose biosensor; DNA/chitosan; direct electron transfer

Introduction

The determination of glucose concentration is very important in clinical, biological and chemical samples, as well as food processing and fermentation. Many techniques have been developed for this purpose. Among the electrochemical techniques, the amperometric biosensors based on electron transfer between an electrode and immobilized glucose oxidase (GOD), which can catalyze the oxidation of glucose, are especially promising. The direct electron transfer of immobilized GOD is regard to the GOD(FAD) to GOD($FADH_2$) conversion, where FAD is flavin adenine dinucleotide. Since the redox center of GOD(FAD) is deeply buried in a glycoprotein shell, DET from the active center of GOD to an electrode surface is very slow. DET of immobilized GOD, has been achieved on platinum or sputtered platinum (Os van et al., 1996), gold (De Taxis Du Poet et al., 1990), glassy carbon (Narasimhan et al., 1986), carbon paste (CP) (Savitri and Mitra, 1998), graphite surface (Ianniello et al., 1982) and colloidal gold (Liu and Ju, 2002) by amperometricand cyclic voltammetric techniques. Recenttly, nanomaterials, such as carbon nanotubes and nanoparticles (Sun et al., 2006; Salimi et al., 2007; Viticoli et al., 2006; Luo et al., 2006; Deng et al., 2008; Sheng et al., 2009; Fu et al., 2009 ; Shan et al., 2009; Liu et al., 2005; Wang et al., 2009), become an attractive material in bioelectrochemistry for the direct electrochemistry of GOD.

The material which could provide an environment similar to that of redox proteins in native systems and give the protein molecules more freedom in orientation, was suggested to reduce the insulating property of the protein shell for the DET and facilitating the electron transfer (Liu and Ju, 2003). Here we report a method for immobilization of GOD on glassy carbon electrodes using a biocompatible DNA/chitosan polyion complex film. It is known that DNA is a stable, low-cost, and easily adaptable molecule, and it is an extremely favorable material used for fabrication novel bio-recognitaion interface. dsDNA has been found to improve electron transfer as an electron wire on electrode surface. On the other hand, chitosan is a polymeric material, biodegradable, non-toxic and has been used as a cholesterol-lowering agent and as a wound-healing product (Illum, 1998). Because of its cationic nature, chitosan can form polyion complex with DNA. DNA/chitosan polyion complex membrane has been used as a support for immobilization of electrocatalytic species-copper ions for a hydrogen peroxide biosensor (Gu et al., 2009). Here we report a GOD electrode showing DET without the need for nanomaterials, which the enzyme was immobilized with a DNA/chitosan biomaterial film. The direct electrochemistry of glucose oxidase and biosensing for glucose based on DNA/chitosan film were studied.

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1 Experimental

1.1 Reagents and chemicals.

Double-stranded DNA (from Herring Sperm, < 5 0 bp) was obtained from Sigma. Chitosan was purchased from Sinopharm Chemcial Reagent Co. All the other chemicals were of analytical grade and used without further purification.

1.2 Preparation of DNA/GOD/chitosan/GC electrode.

A glassy carbon (GC) electrode (diameter 3 mm; Lanlike Chemistry and Electron High Technology Co.) was polished with 0.05 μm alumna slurry, rinsed with water, sonicated in water for 2 min, and dried. The DNA /GOD/chitosan immobilized electrode was prepared as described below. 10 μL of 1 mg/mL dsDNA, 10 μL of 0.5 mg/mL chitosan and 10 μL of 1 mg/mL GOD were placed on electrode surface. The electrode was kept for one day under a 500 mL beaker at room temperature. The electrode was stored in the refrigerator at 4°C when not in use.

1.3 Electrochemical measurements

All electrochemical experiments were carried out with a three-electrode system comprising a DNA/GOD/chitosan /GC electrode as a working electrode, an Ag/AgCl (sat. KCl) reference electrode and a platinum wire auxiliary electrode (1 mm diameter). A 100 mol/L phosphate buffer (pH 7, 10 mL) was used as an electrolyte solution. Cyclic voltammetry (CV) was performed with an electrochemical analyzer (CHI823, Shanghai Chenhua Instrument, Inc. China). All measurements were done at room temperature.

1.4 Scanning electron microscope analysis

The SEM analysis of DNA/GOD/chitosan polyion complex membrane was performed with a JEOL-6480LV microscope operating at 15.0 kV. DNA/GOD/chitosan polyion complex membrane was prepared as follows: 10 μL of 1 mg/mL dsDNA, 10 μL of 0.5 mg/mL chitosan and 10 μL of 1 mg/mL GOD were placed on a glass slide, and allow to dry in air for one day. Prior to SEM analysis, ca. 10 nm of carbon film was coated onto the samples with ion coater. The DNA/chitosan film was also prepared as a control in a similar manner.

2 Results and discussion

2.1 Structures of DNA/GOD/chitosan film surface

Figure 1 shows the SEM views of the DNA/chitosan film and DNA/GOD/chitosan film surfaces. The DNA/chitosan film (Fig. 1a) shows a smooth surface. In contrast, with immobilization of enzymes, DNA/GOD/chitosan film (Fig. 1b) shows a disperse-distributed small spots and rough surface.

2.2 Electrochemistry of DNA/GOD/chitosan/GC electrode

Figure 2 shows cyclic voltammograms (CV) of DNA/chitosan/GC, DNA/GOD/chitosan/GC and GC electrodes in N_2-saturated phosphate buffer solution. A pair of well-defined, quasi-reversible redox peaks redox peaks at the DNA/GOD/chitosan modified electrode was obtained (Fig. 2a). The formal potential ($E^{\circ\prime}$) calculated by averaging the cathodic and anodic peak potentials was estimated as about (–471 ± 5) mV (vs. Ag/ACl in saturated KCl). In comparison with the CV curve of the DNA/chitosan modified electrode (Fig. 2b) and free GC electrode (Fig. 2c), it can be concluded that the redox waves should be ascribed only to GOD, which is characteristic of reversible electron transfer process of redox active center (FAD) in the GOD. Thus, a direct electron transfer of GOD in the DNA/chitosan film has been achieved successfully.

The cyclic voltammograms of DNA/GOD/chitosan modified GC electrode at various scan rates were investigated (Fig. 3). The peak-to-peak separation and the linear relationship between peak current and scan rate indicated that the redox process of GOD in the film was a reversible and surface-confined process. The electron transfer rate constant (k_s) has been estimated from the peak potential separation value using the model of Laviron. Taking a charge transfer coefficient α of 0.5, the electron transfer rate constant of GOD at the DNA/GOD/chitosan modified electrode was (0.91 ± 0.5) sec^{-1} .

It is well known that the direct electrochemistry of GOD is a two-electron coupled with two-proton reaction, which undergoes a redox reaction as follows:

$$\text{GOD-FAD} + 2e^- + 2H^+ \longleftrightarrow \text{GOD-FADH}_2 \quad (1)$$

Therefore, the pH value of the solution has influence on the electrochemical behavior of GOD at

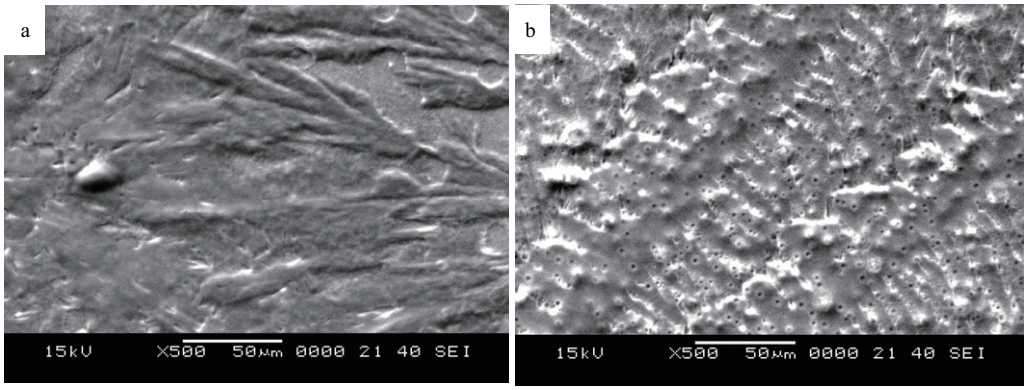

Fig. 1 SEM images of DNA/chitosan film (a) and DNA/GOD/chitosan film (b).

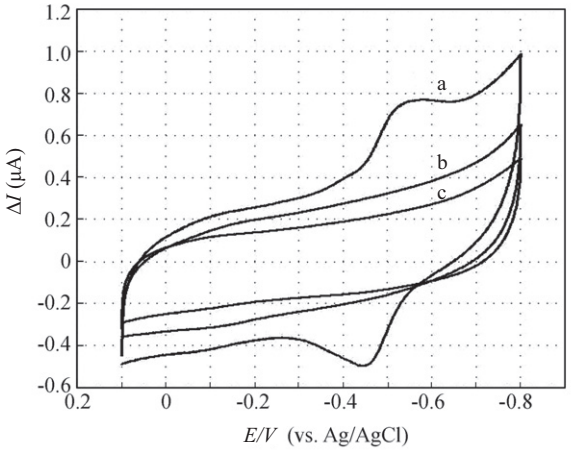

Fig. 2 Cyclic voltammograms of DNA/GOD/chitosan/GC (a), DNA/chitoan/GC (b) and GC (c) electrodes in N_2-saturated phosphate buffer solution (pH 5.5).

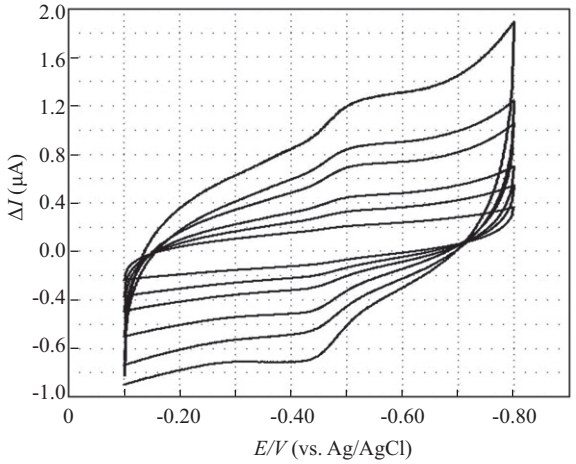

Fig. 3 Cyclic voltammograms of DNA/GOD/chitosan modified glassy carbon electrode at different scan rates from inside out 10, 20, 30, 50, 75, 100 mV/sec in N_2-saturated phosphate buffer solution (pH 5.5).

the DNA/GOD/chitosan/GC electrode. Cyclic voltammograms of the DNA/ GOD/chitosan/GC electrode in the solutions with different pH values were obtained. Stable and well-defined cyclic voltammograms can be observed in the pH range of 5.5–8.0. An increase in solution pH caused a negative shift of both cathodic and anodic peak potentials. The formal potential of the DNA/GOD/chitosan/GC electrode depended linearly on the pH value in the range of 5.5–8.0 with a slop of –39.5 mV/pH (r= 0.998), which was close to the theoretical value of –58.0 mV/pH according to the reaction shown in Reaction (1).

The effect of the dissolved oxygen on the electrochemical behavior of the DNA/GOD/chitosan/GC electrode was investigated. The reduction peak current of the DNA/GOD/chitosan/GC electrode in air-saturated phosphate buffer was larger than that in deoxygenated phosphate buffer and the oxidation peak current was in reverse. It confirms that oxygen dissolved in the solution was reduced at the DNA/GOD/chitosan modified electrode. When glucose was added into air-saturated phosphate buffer, the reduction peak currents at the DNA/GOD/chitosan/GC electrode decreased gradually

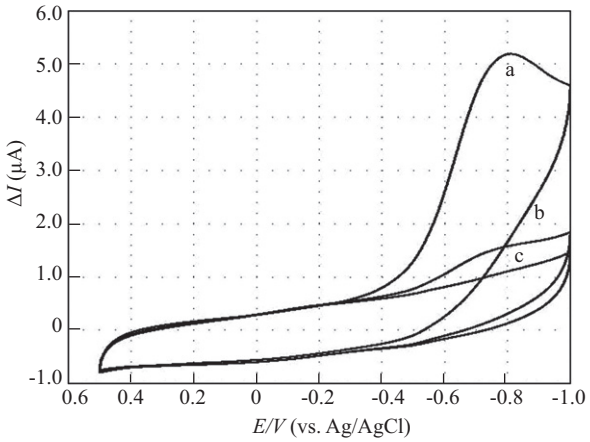

Fig. 4 Cyclic voltammograms of DNA/GOD/chitosan modified glassy carbon electrode in air saturated 0.1mol/L phosphate buffer in the presence of various glucose concentrations. (a) 0 mmol/L, (b) 0.5 mmol/L; (c) 1.5 mmol/L.

with the increase of the concentration of glucose, as shown in Fig. 4. According to the following enzyme-catalyzed reaction,

$$\text{Glucose} + \text{GOD(FAD)} \longrightarrow \text{Gluconolactone} + \text{GOD(FADH}_2\text{)} \quad (2)$$

$$\text{GOD (FADH}_2\text{)} + \text{O}_2 \longrightarrow \text{GOD (FAD)} + \text{H}_2\text{O}_2 \quad (3)$$

Thus, the addition of glucose restrained the electrocatalytic reaction and led to the decrease of reduction current.

2.3 Glucose determination

Figure 5a shows the steady-state current response of glucose for the constant electrode potential of –0.45 V. As shown, during the successive addition of 0.1 mmol/L of glucose, a well response was observed. The plot of response current versus glucose concentration was shown in Fig. 5b. The calibration range of glucose concentration was from 0.04 to 15 mmol/L. The linear response range of the sensor to glucose concentration was from 0.04 to 0.228 mmol/L with a correlation coefficient of 0.997 and a detection limit of 0.04 mmol/L at a signal-to-noise ratio of 3. An extremely attractive feature of the DNA/GOD/chitosan modified GC electrode, fast response time (i.e., 10 sec) and its highly stable amperometric response toward glucose. Furthermore, the sensitivity of the DNA/GOD/chitosan/GC electrode was calculated to be 0.48 μA/(mmol/L). For glucose concentration above 10 mmol/L, a response plateau was observed, showing characteristics of the Michaelis–Menten kinetic mechanism. The Michaelis–Menten constant of the system (K_M) in this work was found to be 6.91 mmol/L, implying that the DNA/GOD/chitosan modified glassy carbon electrode exhibits a higher affinity for glucose.

The effect of the possible interfering species, such as uric acid and ascorbic acid, on glucose detection was also investigated. No obvious interference of 0.1 mmol/L uric acid and 0.1 mmol/L ascorbic acid was observed for the response of 0.4 mmol/L glucose. This indicated that the DNA/GOD/chitosan/GC electrode had a good selectivity. The reproducibility of the glucose biosensor

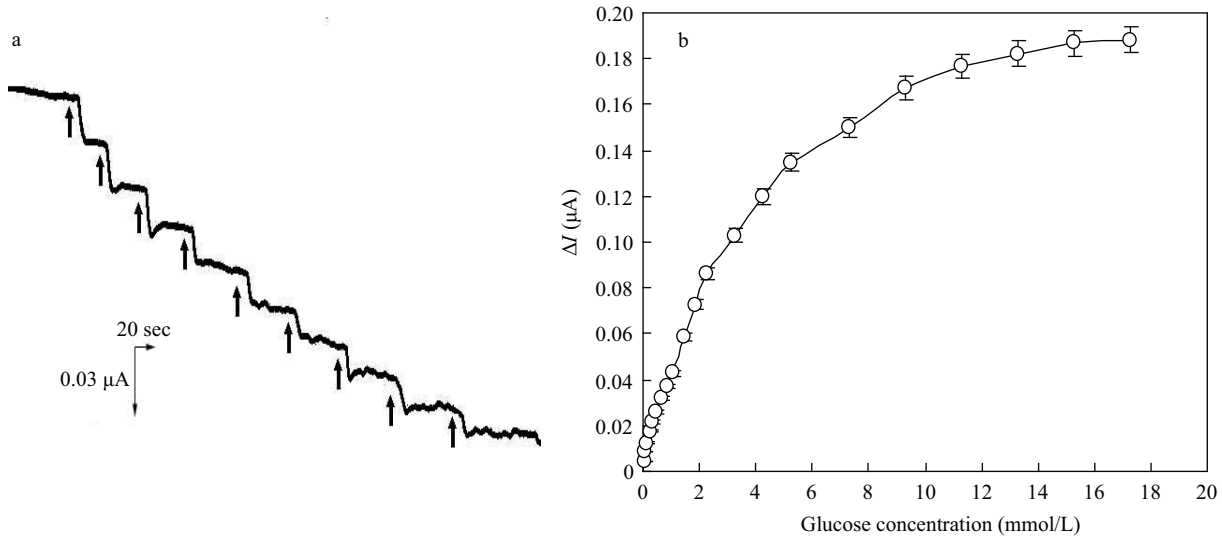

Fig. 5 Steady-state current response of successive addition of 0.1 mmol/L of glucose for the constant electrode potential of –0.45 V (a) and the decrease of cathodic currents vs. glucose (b).

was also investigated by detecting 0.1 mmol/L glucose successively for ten times, the relative standard deviation (RSD) is 3.61%, demonstrating the biosensor has a good reproducibility.

3 Conclusions

GOD can be effectively immobilized on DNA/chitsoan modified GC electrode to produce a fast direct electron transfer. The direct electrochemistry of GOD was performed successfully and the immobilized GOD retained its bioactivity. Based on the decrease of electrocatalytic response, a novel glucose sensor has been developed.The DNA/GOD/chiostan/GC electrode displayed a high sensitivity 0.48 μA/(mmol/L) and a wide linear range up to 0.228 mmol/L for glucose response. Additionally, the biosensor based on DNA/GOD/chiostan/GC electrode exhibited good stability and selectivity.

Acknowledgments

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