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Direct Electron Transfer of Glucose Oxidase and Carbon Nanotubes Entrapped with Biocompatible Organic Materials

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Efficient electron transfer between redox enzymes and electrodes is essential for enzyme-based biosensors, biofuel cells, and bioelectronic devices. Generally glucose oxidase (GOx) requires mediators for electrical communication with electrodes because the redox center of GOx is deeply buried in the insulating protein shell. In the present work, direct electron transfer (DET) between GOx and electrodes was attempted. GOx and carbon nanotubes (CNTs) were immobilized on a glassy carbon (GC) electrode by using biocompatible polymer, chitosan (CHI). Cyclic voltammograms revealed that the CHI/GOx/CNT-GC electrode showed a pair of well-defined redox peaks in 0.1 M phosphate buffer solution (pH 7.0) saturated with argon. Under the same conditions, no redox peak was observed in the absence of CNTs. The formal redox potential was −450 mV (vs. Ag/AgCl), which agreed well with that of $FAD/FADH_2$, the redox center of GOx. This result clearly shows that the DET between the GOx and the electrode was achieved. The use of thin CNTs significantly improved the DET efficiency of the GOx. It was found that the GOx immobilized on the electrode retained catalytic activity for the oxidation of glucose.

Keywords Carbon nanotubes; chitosan; direct electron transfer; glucose oxidase

Introduction

Considerable attention has been attracted by enzyme-based biosensors, biofuel cells, and bioelectronics for which the efficient electrical communication of redox enzymes with electrodes is essential [1–3]. In this respect, the direct electron transfer (DET) is desirable because it does not require any mediators between enzymes and electrodes,

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eliminating problems related to mediator stability, selectivity, and mass transfer limitations [2,3]. In addition, DET-based enzyme electrodes theoretically function at a potential range that is close to the redox potential of the enzyme itself [4]. A number of enzymes were found to be capable of DET, but it is still difficult to promote DET with redox enzymes, the active center of which is deeply located in the insulating protein shell. A typical example is glucose oxidase (GOx), which is a redox enzyme capable of oxidizing glucose using oxygen. Because the redox center of GOx, flavine adenine dinucleotide (FAD) is buried within the protein shell (about 13 Å), the DET rate between the active site and the electrode surface is usually low [4,5]. In recent years, at electrodes modified with nanomaterials such as carbon nanotubes (CNTs), metal nanoparticles, and conducting nanofibers, the DET of the glucose-oxidizing enzyme was accomplished [6–8]. Particularly, CNTs have become excellent nanomaterials for promoting the DET between redox enzymes and electrode surface because of their high surface area, electrical conductivity, good chemical and thermal stability [9–11].

Chitosan (CHI) is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). This natural polymer has been recently explored as electrode coating materials due to its attractive properties such as membrane-forming ability, good adhesion, and high mechanical strength [12,13]. In addition, chitosan is biocompatible and nontoxic, which enables it to become excellent coating materials for GOx electrodes, considering that GOx electrodes are mainly used for medical applications: biosensors for the quantitative determination of glucose in blood and biofuel cells for the power generation of implanted medical devices [14]. In this study, we fabricated DET-based glucose oxidase electrodes using CNTs and chitosan. The CHI/GOx/CNT electrode was electrochemically analyzed in an argon or oxygen-saturated phosphate buffer containing different concentrations of glucose. The direct electron transfer between the entrapped GOx and the electrode surface was investigated by cyclic voltammetry (CV). Effects of the size and amount of CNTs on the DET were also examined in the present work.

Experimental

Materials

Glucose oxidase from *Aspergillus niger* (Type X-S, 147000 units/g solid) and flavine adenine dinucleotide (FAD) were purchased from Sigma (USA). Chitosan (high molecular weight) was obtained from Aldrich (USA). A chitosan solution (0.5 wt%) was prepared by dissolving chitosan in a sodium acetate buffer (0.1 M, pH 4.5). Multi-walled carbon nanotubes, CM95 (>95 wt% purity, 10–15 nm in diameter) and CMP (>95 wt% purity, 4–12 nm in diameter) were purchased from Iljin Nanotech Co. Ltd. (Seoul, Korea) and used without any purification. The CNTs were dispersed in distilled water with ultrasonication for 5 min. A phosphate buffer solution (0.1 M, pH 7.0) was employed as supporting electrolyte.

Fabrication of CHI/GOx/CNT-GC Electrode

A glassy carbon electrode (disk diameter: 2 mm ± 0.2 mm, Princeton Applied Research, USA) was polished on a polishing cloth with 0.05 μm alumina slurry,

and then washed with methanol and distilled water. The CNT suspension (2 μL, 5 mg/mL), GOx solution (5 μL, 30 mg/mL), and chitosan solution (5 μL, 5 mg/mL) were sequentially dripped onto the GC electrode surface and then dried in a desiccator at room temperature.

Analysis

The multi-walled carbon nanotubes were observed using a scanning electron microscope equipped with an energy dispersive spectrometer (Hitachi S-3000N, Japan). Cyclic voltammograms were obtained in a phosphate buffer (0.1 M, pH 7.0) saturated with argon or oxygen at room temperature. A potentiostat (Gamry G750, USA) was used with a conventional three-electrode configuration: a glassy carbon electrode as a working electrode, a platinum wire as a counter electrode and a Ag/AgCl (saturated KCl) electrode as a reference. Potentials in this work are reported versus Ag/AgCl unless indicated.

Results and Discussion

Glucose oxidase contains FAD as a prosthetic group, which is responsible for redox reactions catalyzed by GOx. During the glucose oxidation by GOx, the FAD of GOx is reduced to $FADH_2$, which is then reoxidized back to FAD by oxygen [15]. The DET of GOx means that the redox center of GOx is reduced or oxidized by electrodes instead of glucose or oxygen. First, FAD was electrochemically examined by cyclic voltammetry. In the Ar-saturated phosphate buffer (0.1 M, pH 7.0) containing FAD, a pair of well-defined redox peaks was observed as shown in Figure 1 (solid curve). The formal potential is −436 mV (vs. Ag/AgCl), which is similar to the reported values of −448 mV [8] and −423 mV [10]. When argon was replaced with oxygen, the cyclic voltammogram was changed: the cathodic peak current

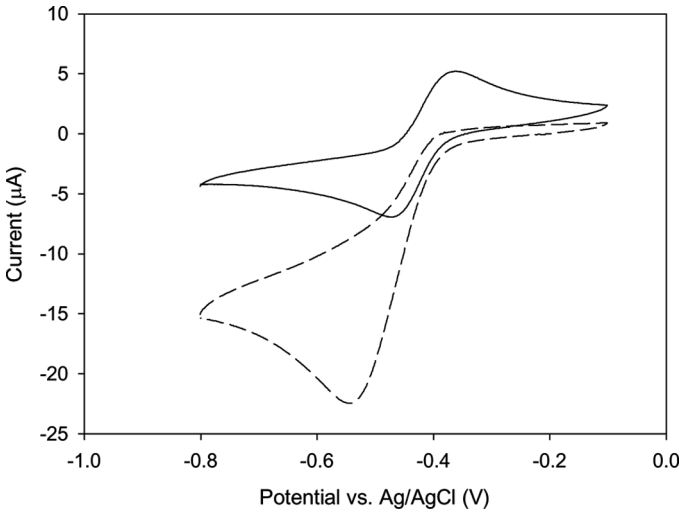


Figure 1. Cyclic voltammograms of FAD (1 mM) dissolved in a phosphate buffer solution (0.1 M, pH 7.0) saturated with argon (solid curve) or oxygen (dashed curve) at a scan rate of 100 mV/s.

significantly increased, accompanied by the decrease of anodic peak current (Fig. 1, dashed curve). This behavior is ascribed to the reduction of oxygen.

In order to check that CNTs are essential for the direct electron transfer of GOx, we prepared the enzyme electrode without CNTs. A glassy carbon (GC) electrode on which GOx was entrapped with chitosan was investigated by cyclic voltammetry under argon. As shown in Figure 2 (curve a), there was no redox peak, demonstrating that the GOx did not electrically communicate with the electrode in the absence of CNTs. The CNTs (CM95) were introduced to achieve the DET of glucose oxidase immobilized on the electrode. Figure 2 (curve b) shows the cyclic voltammograms obtained using the CHI/GOx/CNT-GC electrode as a working electrode under the same conditions. A pair of redox (reduction and oxidation) peaks was observed and the formal redox potential was −450 mV (vs. Ag/AgCl). This value is in agreement with that of FAD/$FADH_2$, revealing that the redox peaks are attributed to the reduction and oxidation reaction of the redox center of GOx immobilized on the electrode surface.

According to Marcus [2,3,16], the DET of redox enzymes, as an electro tunneling mechanism, depends on three major factors: the enzyme structure, specifically the location of the redox center within the protein, the enzyme orientation, and the electron transfer distance. Therefore, an efficient electron transfer between redox enzymes and electrodes can be only accomplished if (i) the active center is close to the protein surface, (ii) the enzyme is oriented with this active site towards the electrode, and (iii) the distance is shorten by the enzyme deformation, by a second redox center, or by modification [3]. In this respect, an electrically conductive matrix, CNTs can reduce the distance from the redox center of GOx to the electrode by acting as a microelectrode surface [4,5]. The three-dimensional network of conductive CNTs can significantly increase the surface area for enzyme immobilization and provide a kind of electrical circuit for the enzyme [4,8,9].

The CHI/GOx/CNT-GC electrode was also examined under the O_2-saturated conditions. When oxygen was supplied into the phosphate buffer instead of argon,

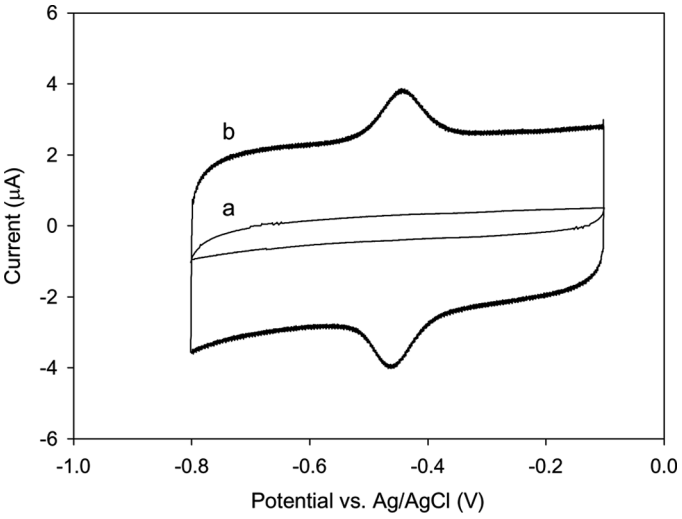


Figure 2. Cyclic voltammograms of the CHI/GOx-GC electrode (curve a) and the CHI/GOx/CNT-GC electrode (curve b) in an argon-saturated phosphate buffer (0.1 M, pH 7.0) at a scan rate of 100 mV/s.

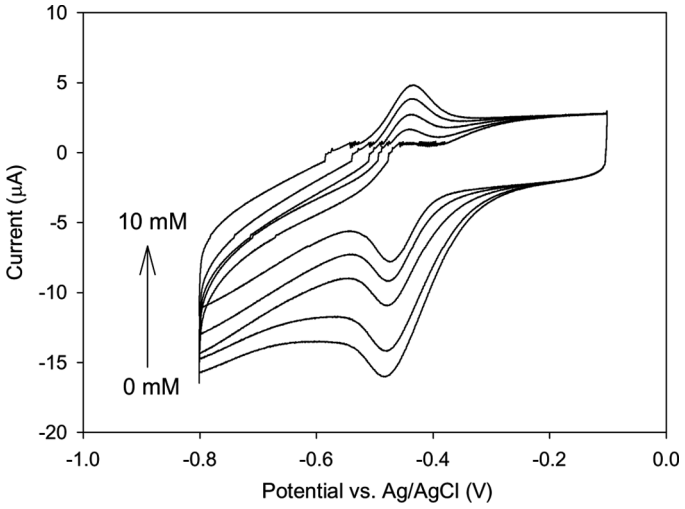


Figure 3. Cyclic voltammograms of the CHI/GOx/CNT-GC electrode in an oxygen-saturated phosphate buffer (0.1 M, pH 7.0) containing 0, 1, 3, 5, and 10 mM glucose at a scan rate of 100 mV/s.

the cathodic current was increased with the anodic current decreased, similarly to Figure 1 (dashed line). This redox curve was shifted towards oxidative current values with increasing the glucose concentration from 0 mM to 10 mM. This is because the GOx immobilized on the electrode catalyzes the reduction of oxygen to hydrogen peroxide with glucose and consequently reduces the concentration of oxygen at the electrode surface. Figure 3 clearly reveals that the GOx immobilized on the fabricated electrode retains its catalytic activity.

Effects of the scan rate on the voltammetric peaks of the CHI/GOx/CNT-GC electrode were also examined. As shown in Figure 4(a), the redox currents increased with increasing the scan rate from 10 mV/s to 1000 mV/s. Figure 4(b) reveals that

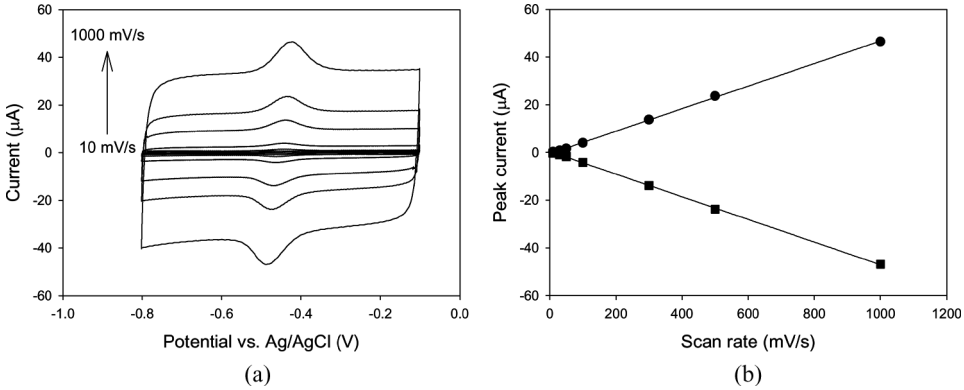
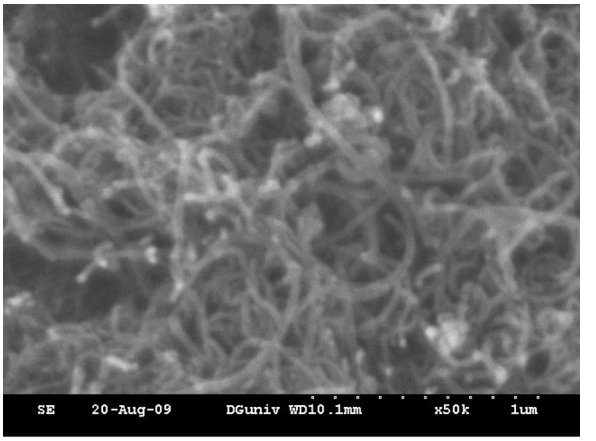


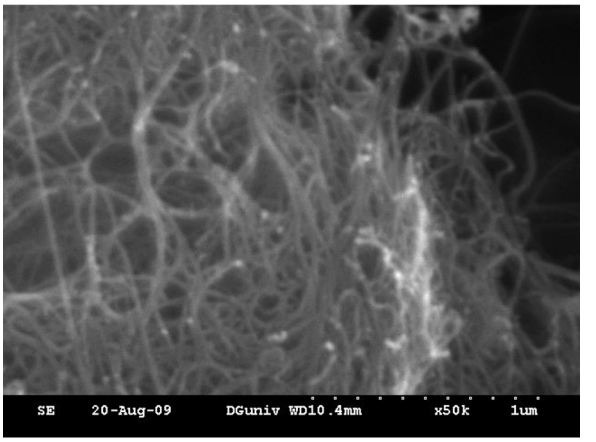
Figure 4. Cyclic voltammograms (a) and peak currents (b) of the CHI/GOx/CNT-GC electrode in an Ar-saturated phosphate buffer (0.1 M, pH 7.0) at a scan rate of 10, 30, 50, 100, 300, 500, and 1000 mV/s.

the peak current is linearly proportional to the scan rate in this range. This is a typical characteristic of surface-confined electrochemical behavior, illustrating that the redox reaction is not a diffusion-controlled process and results from the surface bound FAD group of the GOx [5].

In order to improve the DET efficiency, were employed different multi-walled carbon nanotubes (CMP), which were 4–12 nm in diameter and thinner than the aforementioned CNTs (CM95). SEM images of the CNTs of two different size (CM95 and CMP) are shown in Figures 5(a) and (b), respectively, confirming that CM95 was thicker than CMP. As shown in Figure 6(a), the CHI/GOx/CMP-GC electrode (dashed curve) significantly increased the redox currents compared to the CHI/GOx/CM95-GC electrode (dotted curve). The substantial increase of the redox current might be due to larger surface areas of the thinner CNTs. The current was further enhanced when the amount of CMP cast on the electrode was increased twice (solid curve). In Figure 6(b), the anodic and cathodic peak currents are plotted against the CNT type and amount.



(a)



(b)

Figure 5. SEM images of CM95 (a) and CMP (b).

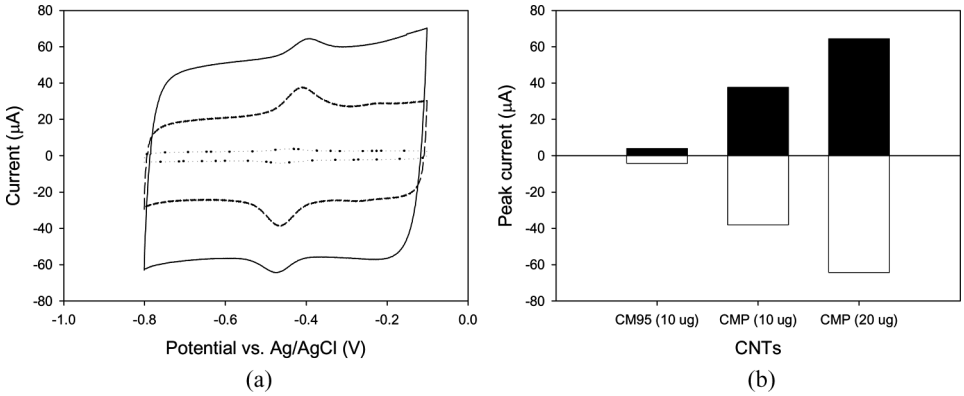


Figure 6. Effects of CNTs on the cyclic voltammograms (a) and peak currents (b) with the CHI/GOx/CNT-GC electrode in an Ar-saturated phosphate buffer (0.1 M, pH 7.0) at a scan rate of 100 mV/s. Different size or amount of CNTs were used: CM95, 10 μg (dotted curve); CMP, 10 μg (dashed curve); CMP, 20 μg (solid curve).

Conclusions

The glucose oxidase electrode was successfully fabricated using multi-walled carbon nanotubes and chitosan, biocompatible organic polymer. With the CHI/GOx/CNT-GC electrode was observed a pair of well-defined redox peaks, which was close to that of FAD, redox center of GOx. This result demonstrates that direct electron transfer between the immobilized GOx and the electrode was achieved. The use of thin CNTs significantly improved the DET efficiency of the GOx. It was found that the GOx entrapped on the electrode with chitosan retained catalytic activity for the oxidation of glucose.

Acknowledgments

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