Effect of Carbon Nanotubes on Direct Electron Transfer and Electrocatalytic Activity of Immobilized Glucose Oxidase

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ABSTRACT: Carbon nanotubes (CNTs) are excellent supports for electrocatalysts because of their large surface area, excellent electronic conductivity, and high chemical and structural stability. In the present study, the activity of CNTs on direct electron transfer (DET) and on immobilized glucose oxidase (GOX) is examined as a function of number of walls of CNTs. The results indicate that the GOX immobilized by the CNTs maintains its electrocatalytic activity toward glucose; however, the DET and electrocatalytic activity of GOX depend strongly on the number of inner tubes of CNTs. The GOX immobilized on triple-walled CNTs (TWNTs) has the highest electron-transfer rate constant, 1.22 s⁻¹, for DET, the highest sensitivity toward glucose detection, 66.11 ± 5.06 μA mM⁻¹ cm⁻², and the lowest apparent Michaelis–Menten constant, 6.53 ± 0.58 mM, as compared to GOX immobilized on single-walled and multiwalled CNTs. The promotion effect of CNTs on the GOX electrocatalytic activity and DET is most likely due to the electron-tunneling effect between the outer wall and inner tubes of TWNTs. The results of this study have general implications for the fundamental understanding of the role of CNT supports in DET processes and can be used for the better design of more effective electrocatalysts for biological processes including biofuel cells and biosensors.

1. INTRODUCTION

Direct electrochemistry of redox enzymes/proteins such as glucose oxidase (GOX) plays an important role in the glucose oxidation reactions in enzyme-immobilized electrodes in microbial and enzymatic biofuel cells. In enzymatic/microbial fuel cells and chemical biosensors, enzymes or microbes are generally immobilized on electrode material surfaces; however, a key issue in such systems is the promotion of ET (DET) with the bare electrode is difficult because of the fact that the redox active flavin adenine dinucleotide (FAD) cofactors, that is, small nonproteinaceous electroactive species, of GOX are deeply buried within electrically well-insulated prosthetic shells. In enzymatic/microbial fuel cells and chemical biosensors, enzymes or microbes are generally immobilized on electrode material surfaces; however, a key issue in such systems is the promotion of ET (DET) with the bare electrode is difficult because of the fact that the redox active flavin adenine dinucleotide (FAD) cofactors, that is, small nonproteinaceous electroactive species, of GOX are deeply buried within electrically well-insulated prosthetic shells. Therefore, considerable efforts have been made to enhance the DET of GOX via the redox mediators and the selected matrix and to develop new and high-performance biofuel cells and enzymatic chemical biosensors. Mediators such as 2-hydroxy-1,4-naphthoquinone, thionin, ferrocene monocarboxylic acid, and methylene blue offer advantages for immobilization of enzymes and enhancement of DET capacity and power output of biofuel cells. In mediated ET using electroactive molecules or mediators to shuttle electrons between the enzyme and the electrode, the maximum cell voltage of biofuel cells is determined by the thermodynamic redox potential of mediators. A more positive redox potential is required to provide the driving force for the ET between the enzyme active center and the electrode for the oxidation biocatalysts, which contributes to cell voltage loss. Another area of intensive research is to use advanced smart carbon materials such as carbon nanotubes (CNTs), carbon black, carbon nanoparticles (NPs), vertically aligned CNTs, and graphene in immobilization and growth of enzymes/proteins and living cells for various biological processes and for electrochemical biosensors and biofuel cells. Among them, CNTs have attracted considerable attention for potential applications such as supporting materials for enzymes owing to their unique electrical conductivity, high chemical stability, biocompatibility, and large surface area. However, it has been shown that the physical and chemical properties of CNTs can influence the adsorption and activity of immobilized enzymes. Pang et al. studied the effect of various carbon materials on the enzyme loading and laccase activity, including fullerene (C-60), multiwalled CNTs (MWNTs), oxidized MWNTs, and graphene oxide and found that the immobilized enzymes have significantly reduced reaction rates as compared to free laccase. This has been attributed to the nanomatrix-induced diffusion limitation on the enzyme activity.

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Recently, we have found that pristine CNTs composed of 2–3 concentric tubes or walls have significantly higher electrochemical activities for O₂ evolution reactions (OERs) as compared with typical single-walled CNTs (SWNTs) and MWNTs. The electrocatalytic activity of CNTs shows a distinct volcano-type curve as a function of the number of walls of CNTs. Similar volcano curves were also observed on Pt and Pd NP-supported CNTs for methanol, ethylene glycol, ethanol, and formic acid oxidation in alkaline solutions and on dye-functionalized CNTs for photoelectrochemical (PEC) water splitting. This indicates that the number of walls or inner tubes of CNTs plays an important role in promoting the ET of the electrochemical redox reaction on the surface of CNTs, and such a promotion effect could be beneficial to the DET of the redox reactions of enzymes/proteins. Here, the effects of quantum properties of CNTs on the DET and electrocatalytic activity of GOX toward glucose oxidation were studied. The results indicate that triple-walled CNTs (TWNTs) facilitate fast DET of GOX and enhance the electrocatalytic activity for glucose sensing, as compared to that on SWNTs and MWNTs.

2. RESULTS AND DISCUSSION

2.1. CNTs and Direct Electrochemistry of GOX/CNT-Modified Glassy Carbon Electrodes. Figure 1 shows the transmission electron microscopy (TEM) micrographs of the CNTs used in this study. CNTs were divided into four groups, depending on the number of walls. CNT-1 mainly consists of SWNTs (79%) with an outer diameter (OD) of 1.97 nm. CNT-2 is dominated by TWNTs (52%) and double-walled CNTs (DWNTs, 25%) with an OD of 3.80 nm. The average number of walls of CNT-3 is seven with an OD of 7.45 nm, whereas CNT-4 is a typical MWNT with an average of 12 walls and an OD of 13.9 nm. CNTs with a small OD prefer to form bundles because of the van der Waals interactions; however, CNTs with a large OD, CNT-3 and CNT-4, are well-dispersed without bundles. After purification, the amount of Fe, Co, Mo, and Ni elements are substantially reduced to less than 100 ppm as confirmed by the inductively coupled plasmaoptical emission spectroscopy analysis. The details of CNTs used in this study can be found elsewhere. The Brunauer–Emmett–Teller (BET) surface area of CNTs is in the range of 271–557 m² g⁻¹. Table 1 summarizes the average number of walls and surface areas of CNTs.

### Table 1. Average OD, Average Wall Numbers (n), and the BET Surface Area (Sₜₐₜ) of CNTs

| Sample  | OD/nm | n  | Sₜₐₜ/m² g⁻¹ |
|---------|-------|----|-------------|
| CNT-1   | 1.97  | 1  | 577         |
| CNT-2   | 3.80  | 3  | 523         |
| CNT-3   | 6.90  | 7  | 539         |
| CNT-4   | 13.8  | 12 | 271         |

Figure 2A shows the cyclic voltammograms (CVs) of the bare glassy carbon (GC) and CNT-modified GC electrodes in an N₂-saturated 0.1 M phosphate-buffered saline (PBS) solution at a scan rate of 50 mV s⁻¹. No reduction or oxidation peaks were observed in the CVs of both bare GC and CNT-modified electrodes. However, the CNT-modified GC electrodes show substantially high background current as compared to bare GC electrodes, indicating the high active sites of CNTs and the formation of well-integrated CNT-modified GC electrodes. In the case of GOX/CNT-modified electrodes, a pair of well-defined and symmetrical redox peaks with equal oxidation and reduction peak heights at about −0.48 V appears (Figure 2B). It is well-known that the cofactor of GOX, existing in two different states, FAD (oxidized form) and FADH₂ (reduced form), can work as an electron acceptor and donor alternatively in redox reactions. The observed redox peaks indicate that FAD from GOX can undergo reversible direct electrochemical oxidation and reduction on the GOX/CNT-modified GC electrodes without the help of the electron-transfer mediators. On the other hand, the CV obtained from the electrode modified with GOX only is featureless, indicating that the DET between the GC electrode and FAD, the active redox center of GOX, is very weak without CNT modification. CNTs have the excellent electron-transport property and high special surface area, which promotes significantly the ET and facilitates the DET process between the redox center of GOX and the GC electrode substrate as shown in this study and also by others. The distinct differences of the electrochemical behavior of CNTs, GOX, and GOX/CNTs as shown in Figure 2 also clearly indicate the immobilization of GOX on CNTs. The immobilization of GOX on CNTs is most likely due to the π=π and σ−σ stacking similar to that observed in the case of polyelectrolyte-functionalized CNTs.

The oxidation and reduction peak potentials are located in the potential range between −0.431 and −0.473 V (vs Ag/AgCl). The formal potential (E°) of the electrodes, calculated by averaging the cathodic and anodic peak potentials, is in the range of 0.449−0.458 V, which is close to the standard potential of FAD/FADH₂ (i.e., −0.460 V in pH 7.0 at 25.8 °C). Similar values were also reported for other GOX-modified GC electrodes. The separation potential of the cathodic and anodic peaks, ΔE_p, depends on the CNTs. The ΔE_p values of GOX/CNT-1, GOX/CNT-2, GOX/CNT-3, and GOX/CNT-4 are 35, 24, 31, and 34 mV, respectively. For the redox reaction on GOX/CNTs, the ΔE_p value shows the lowest value at the GOX/CNT-2-modified GC electrode, 0.024 V, proving a fast electron-transfer process on GOX-immobilized CNT-2. Periasamy et al. studied the direct electrochemistry of GOX at gelatin- and N,N-dimethylformamide (DMF)-dispersed MWNTs and GMWNT- and DMWNT-modified GC electrodes, and observed that the difference of ΔE_p...
for GOx/GMWNT and GOx/DMWNT is 14 mV ($\Delta E_p$ was 47 mV for GOx/GMWNT and 33 mV for GOx/DMWNT). This is close to the difference of $\sim$10 mV of the $\Delta E_p$ values between GOx/CNT-2 and GOx/CNT-1 and between GOx/CNT-2 and GOx/CNT-4. The experiments were repeated several times, and the peak potentials were generally reproducible with an SD of $\sim$7%. The electrochemical parameters for the DET of GOx at different CNT-modified GC electrodes are given in Table 2.

The reversibility of DET of GOx on CNT-modified GC electrodes was investigated by CVs at different scan rates under N2-saturated conditions (Figure 3). With the increase of the scan rates, the anodic peak potentials are shifted to a more positive potential, whereas the cathodic peaks are shifted towards more...
negative potential with no change in the formal potential, \( E^\circ \). The linear increase in the peak current with scan rates (the insets of Figure 3) demonstrates that the redox reaction of FAD/FADH\(_2\) involved in the GOX adsorbed on the CNT supports is a surface-controlled process but not a diffusion-controlled process.\(^7,43\) The ET rate constant (\( k_s \)) can be calculated from \( \Delta E_p \) using the Laviron equation for a surface-controlled electrochemical system\(^45\) with a charge transfer coefficient (\( \alpha \)) of 0.5. The \( k_s \) values of GOX/CNT-modified GC electrodes were determined to be 0.984 s\(^{-1}\) for GOX/CNT-1, 1.22 s\(^{-1}\) for GOX/CNT-2, 1.064 s\(^{-1}\) for GOX/CNT-3, and 1.004 s\(^{-1}\) for GOX/CNT-4. The \( k_s \) value obtained on the GOX/CNT-2-modified electrode is also higher than 1.16 cm s\(^{-1}\) reported by Deng et al. for the redox reaction of GOX on the undoped CNTs but lower than 1.56 s\(^{-1}\) on boron-doped CNTs.\(^7\) The high \( k_s \) values mean faster electron-transfer rate between the electrode and the redox-active center of the enzyme.\(^7\) The significant dependence of the \( k_s \) on CNT supports indicates that the DET of GOX depends on the number of walls of CNT supports, similar to that observed for alcohol oxidation and OERs in alkaline solutions.\(^31,32\)

2.2. Electrocatalytic Activity of GOX/CNT-Modiﬁed GC Electrodes. Figure 4 shows the electrochemical behavior of the GOX/CNT-modiﬁed GC electrodes in N\(_2\)- and O\(_2\)-saturated PBS solutions. There are two clearly separated reduction peaks for the reaction on the GOX/CNT-modiﬁed GC electrodes in O\(_2\)-saturated PBS solutions, except that on GOX/CNT-4. The first one around −0.35 V is most likely associated with the reduction reaction of O\(_2\) by CNTs. This is consistent with an early study that except MWNTs, pristine CNTs have a considerable electrochemical activity for the O\(_2\) reduction reaction (ORR).\(^46\) This appears to be supported by the observation of a broad reduction peak at −0.4 V for the reaction on GOX/CNT-4-modiﬁed GC electrodes, which is consistent with the observed oxygen reduction shoulder near −0.4 V on gelatin- and DMF-dispersed MWNT-modiﬁed GC electrodes.\(^44\) The second one around −0.45 V is the O\(_2\) reduction peak by GOX. However, the reduction peak current of the GOX/CNT-modiﬁed GC electrodes increases signiﬁcantly in O\(_2\)-saturated PBS solution, as compared with that recorded under N\(_2\)-saturated conditions, with subsequently reduced oxidation current. This implies that GOX immobilized on CNTs catalyzes O\(_2\) reduction effectively because of the fact that O\(_2\) is a cosubstrate of GOX. The direct electrochemistry of GOX is a two-electron two-proton process and undergoes a redox reaction between GOX(FAD) and GOX(FADH\(_2\)). FADH\(_2\) could be oxidized by O\(_2\) at the GOX/CNT-modiﬁed GC electrodes, which causes the increase of the reduction peak current of GOX (FAD) to O\(_2\), catalyzing the decrease of the oxidation peak current of GOX (FAD) to O\(_2\) (Reaction 1).

\[
\text{GOX(FAD)} + 2e^- + 2H^+ \leftrightarrow \text{GOX(FADH}_2\text{)} \\
\text{GOX(FADH}_2\text{)} + O_2 \rightarrow \text{GOX(FAD)} + H_2O_2
\]

This is consistent with other studies on different GOX-immobilized electrodes.\(^6,7\)

The reduction peak currents at the GOX/CNT-modiﬁed GC electrodes decrease gradually with the addition of glucose in an O\(_2\)-saturated PBS solution (Figure 5). This indicates that GOX immobilized on CNTs retains its bioelectrocatalytic activity to glucose because of the fact that glucose is the natural substrate of GOX.\(^5\) The presence of glucose will lead to an enzyme-catalyzed reaction and decrease the concentration of the oxidized form of GOX that is, GOX(FAD), and thus the decrease of the reduction current.

\[
\text{GOX(FAD)} + \text{glucose} \rightarrow \text{GOX(FADH}_2\text{)} + \text{gluconolactone}
\]

To further investigate the effect of different CNT supports on the current response of the GOX/CNT-based glucose biosensor,
the amperometric responses of GOx/CNT-modified GC electrodes have been determined on successive injections of glucose to an O2-saturated 0.1 M PBS solution under an applied potential of −0.48 V versus Ag/AgCl. As shown in Figure 6, the reduction currents decrease with the increase of the glucose concentration. On the basis of the decrease of the reduction current, the concentration of glucose can be detected. Therefore, the GOx/CNT-modified GC electrode can be used as a glucose biosensor. Figure 7 shows the corresponding plots of the reduction current measured at −0.48 V at GOx/CNT-modified GC electrodes versus the glucose concentration. The reduction currents of GOx/CNT-modified GC electrodes change linearly with the concentration of glucose up to 4.31–5.44 mM, with a correlation coefficient greater than 0.994. The sensitivity of the
GOX/CNT-modified GC electrodes is calculated to be 37.38 ± 5.75, 66.11 ± 5.06, 35.75 ± 2.23, and 36.77 ± 4.88 µA mM⁻¹ cm⁻² on GOX-immobilized CNT-1-, CNT-2-, CNT-3-, and CNT-4-modified GC electrodes, respectively. The sensitivity obtained on GOX/CNT-4 is close to 40.14 µA mM⁻¹ cm⁻², which is reported on GOX immobilized on undoped MWNTs. Similar to kₚ, the highest sensitivity is obtained on GOX immobilized on TWNTs, GOX/CNT-2.

The apparent Michaelis–Menten constant (Kᵈ app) is an indicator of the enzymatic activity of the immobilized GOX and can be estimated by the Lineweaver–Burk equation:

\[
\frac{1}{i_S} = \frac{1}{i_{max}} + \frac{K_{app}}{i_{max}} \times \frac{1}{C_{glucose}}
\]

where iₜ is the steady-state response current after the addition of the substrate, iₘ is the maximum current under saturated substrate conditions, and C glucose is the bulk glucose concentration. Under the conditions of this study, and K app of GOX/CNT-modified GC electrodes is estimated to be in the range of 6.53–11.49 mM. The low K app value implies a strong ability of substrate binding and high enzymatic activity of the immobilized GOX. Consistent with the high transfer rate and high sensitivity, the GOX/CNT-2-modified GC electrode exhibits the smallest K app value, 6.53 ± 0.58 mM, indicating that the GOX immobilized on the TWNTs maintains an excellent catalyst activity and exhibits fabulous affinity toward glucose. Table 3 summarizes the sensitivity and K app values of GOX/CNT-modified GC electrodes.

2.3. Role of the Number of Walls of CNT Supports.

Before the discussion of the role of the number of inner tubes on the electrochemical behavior of GOX immobilized on CNTs, it is necessary to clarify the effect of the surface areas and residual metal impurities of CNTs used in this study. In catalysis, the electrocatalytic activity of catalysts is generally normalized with the surface area of the catalytic NPs to eliminate or avoid the effect of the size of the catalysts. It is well-known that the size of supported catalytic NPs plays a determining role in their activity and stability, and the catalytic specific activity usually increases with the decreasing NP size because of the rising number of low-coordinated metal atoms and/or defects as the catalytically active sites. In this study, the BET surface area of CNTs is close for CNTs with the number of layers from 1 to 7: 577, 523, and 539 m² g⁻¹ for CNT-1, CNT-2, and CNT-3 (see Table 1), respectively. Only in the case of CNTs with 12 layers (CNT-4, MWNTs), the surface area decreased significantly to 271 m² g⁻¹. This indicates that if the surface area of CNT substrates is a dominant fact in the electrochemical behavior of GOX/CNTs, the electrochemical activity of GOX/CNTs should be similar at least for GOX immobilized on CNT-1, CNT-2, and CNT-3. However, this is not the case, as shown in this study, and the electrocatalytic activity of GOX/CNTs depends strongly on CNT-1, CNT-2, and CNT-3, and the best results were obtained on GOX/CNT-2, even though the surface area of CNT-2 is actually slightly lower than that of CNT-1. Despite the fact that the surface area of CNT-1 is much higher than that of CNT-4, their kₚ sensitivities, and K app values are very close (Tables 2 and 3). Therefore, the surface area is not a dominant factor in the DET and electrocatalytic activity of GOX/CNT-modified GC electrodes. The amounts of metallic impurities of CNT supports after purification are less than 100 ppm. As shown in Figure 2A, pristine CNTs show no redox peaks in 0.1 M PBS solution, indicating that such low levels of metallic impurities in CNTs do not contribute to the electrochemical properties of GOX.

As shown by Marcus and Sutin, the ET rate exponentially depends on the distance of the closest approach between an electron donor and acceptor and is negligible for distances beyond 2 nm. This indicates that the DET of FAD active sites deeply embedded (~1.5 nm) within the protein to the electrode surfaces would be very difficult. This is supported by the featurelessness of the CV curves of GOX-modified GC electrodes without immobilization of CNTs (Figure 2B). It has been well-known that CNTs promote the DET to GOX in alkaline solutions, showing a distinct volcano-type curve as a function of the number of inner tubes or walls. The intrinsic activity of pristine CNTs has been evidently demonstrated by the KCN blockage experiments, in which cyanide ions, CN⁻, strongly coordinate with transition metals such as Co, Fe, and Ni in the axial position, thus completely blocking and poisoning...

Table 3. Parameters Estimated from the Amperometric Response for the Different GOX/CNT-Modified GC Electrodes in Glucose Solutions

| electrode   | K app (mM) | sensitivity (µA mM⁻¹ cm⁻²) | linear range (mM) | linear regression equations |
|-------------|------------|-----------------------------|-------------------|----------------------------|
| GOX/CNT-1   | 9.24 ± 1.5 | 37.38 ± 5.73                | 0.25–5.44         | y = 4.8828x + 0.6025 (R² = 0.9971) |
| GOX/CNT-2   | 6.53 ± 0.58| 66.11 ± 5.06                | 0.25–4.99         | y = 8.5424x + 0.7412 (R² = 0.9948) |
| GOX/CNT-3   | 9.01 ± 1.45| 35.75 ± 2.23                | 0.25–4.31         | y = 4.5977x + 0.2929 (R² = 0.9973) |
| GOX/CNT-4   | 11.49 ± 3.01| 36.77 ± 4.88                | 0.25–4.99         | y = 4.3751 + 1.6219 (R² = 0.9936) |
the metallic impurities on the side walls of CNTs. In the presence of KCN, CNTs show distinctive activity volcano curves, which are identical to those observed on the pristine CNTs in the absence of KCN.46 This evidently demonstrates that the inner tubes of CNTs play a significant role in the enhancement of the charge transfer of the reactions such as OER, ORR, and HER via the electron-tunneling effect between the outer and inner walls.46

The strong promotion effect of inner tubes on the electrocatalytic activity of CNTs has also demonstrated the electro-oxidation of alcohols on Pt and Pd NPs supported on CNTs, with highest activities observed on Pt and Pd NPs supported on CNTs with 2−3 walls.32−34 As shown in Figure 8, the $k_s$ and sensitivity values show distinctive volcano curves, whereas the $K_{\text{app}}$ values exhibit a reversed volcano curve as a function of the number of walls, identical to that observed for alcohol oxidation and OERs in alkaline solutions.31−33 A high $k_s$ value of 1.22 s$^{-1}$ obtained for the DET of GOX/CNT-2 indicates the fast ET for the direct electrochemistry of GOX immobilized on TWNTs, as compared to that immobilized on conventional SWNTs and MWNTs. The observation of the distinguished volcano-type curves of the electrocatalytic activity of GOX/CNTs in this study and that of pristine CNTs and Pt and Pd NPs supported on CNTs as a function of number of inner tubes or walls, despite the significant differences in the electrochemical systems, clearly indicates the existence of the inherent electrocatalytic activities of CNTs. The promotion effect of inner tubes has also been demonstrated most recently on the dye-functionalized CNTs for PEC water splitting, in which the dye-functionalized DWNTs and TWNTs show a much higher PEC activity for water splitting as compared to that of SWNTs and MWNTs.35 In other words, the promotion effect of CNTs via the electron-tunneling mechanism is an intrinsic property of CNTs with a defined number of walls. Thus, the reason for the observation of the highest DET and electrocatalytic activity of GOX immobilized on CNT-2, a mixture of TWNTs and DWNTs, is most likely the facile charge-transfer process via the electron tunneling between the outer wall and inner tubes of CNT-2 under the influence of an electrochemical driving force, which is consistent with previous studies.31−34,46

Figure 9 shows schematically the DET of the redox-active center of GOX and CNTs facilitated by the electron tunneling between the outer wall and inner tubes of CNTs. Similar to the electrochemical reactions of OER on CNTs,31,46 such effective charge or ET as described above would not be possible for the GOX supported on SWNTs and GOX/CNT-1 and diminishes as the number of walls increases because of the significantly reduced polarization driving force or dc bias across the walls or layers of CNTs for the ET between the outer wall and inner tubes. Thus, the ET efficiency of the redox reaction of GOX immobilized on CNT-2, a mixture of DWNTs and TWNTs, is much higher when the ET is between the outer wall and inner tubes as compared to that on SWNTs and MWNTs, GOX/CNT-1, and GOX/CNT-4.

As pointed out by Chen et al., the structural factors such as the number of layers, size, and chirality, and layer stacking modes can significantly alter the heterogeneous electron-transfer efficiency at sp$^2$ nanocarbons such as graphene and CNTs.53,54 CNTs act as electrical conducting nanowires for the fast DET between the active sites in GOX and substrate electrodes. The electron tunneling or transfer efficiency between CNTs and the GOX active sites depends on the density of states (DOS) and the layers of CNTs. For SWNTs, the DOS is rather low near their Fermi levels, and therefore, the ET efficiency is very low. Increasing the wall numbers would increase the DOS near the Fermi level, which would increase the ET efficiency. On the other hand, however, the increase of wall numbers (thickness) of CNTs could increase the ET distance, which could lower the ET efficiency. Thus, CNTs with 2−3 walls may provide an optimized balance between these two effects. This also explains the observed high electrocatalytic activity of CNT-2 toward glucose.

3. CONCLUSIONS

In the present study, we studied in detail the effect of CNT supports on the DET and electrocatalytic activities of GOX in 0.1 M PBS solution. A pair of well-defined and fundamentally reversible redox peaks was observed, indicating an excellent DET between the redox centers of GOX and GC electrodes without the help of the electron-transfer mediators or metallic NPs to contact the FAD active centers. Furthermore, the GOX immobilized by the CNTs still maintained its biocatalytic activity toward glucose. However, the direct electrochemistry and electrocatalytic activity of GOX depend strongly on the nature of CNT supports, showing distinct volcano curves as a function of the number of walls. GOX immobilized on CNT-2, a mixture of TWNTs and DWNTs, is most effective in promoting the DET and electrocatalytic activity toward glucose, showing the highest $k_s$ value and sensitivity and the lowest $K_{\text{app}}$. The excellent promotion effect of CNT-2 is ascribed to the fast DET via the efficient electron tunneling between the outer wall and inner tubes of CNTs. The results demonstrate that the DET and electrocatalytic activities of GOX immobilized on CNTs can be manipulated by the quantum properties of CNT supports.
has general implications for the fundamental understanding of the role of CNT supports in the DET or ET processes, and the results can be used for the better design of biocatalysts for biofuel cell and biosensor applications. For the development of practical biocatalysts, the addition of electrocatalysts would be beneficial to further increase the activity and sensitivity. However, as shown in the present study, the use of CNT supports with 2–3 inner tubes would significantly enhance the activity and sensitivity of the electrocatalysts in applications such as biofuel cells and glucose biosensors.

4. EXPERIMENTAL SECTION

4.1. Materials and Solutions. Glucose oxidase (GOX, EC 1.1.3.4, 100–250 U/mg, type X-S from Aspergillus niger), D-(-)-glucose, DMF, sodium phosphate monobasic, and sodium phosphate dibasic were purchased from Sigma Aldrich. Nafion solution (5 wt % in isopropanol and water) was purchased from DuPont Inc. Four CNTs with different number of walls were purchased from Nanostructured & Amorphous Materials Inc., USA. Deionized double-distilled water (18.6 MΩ, Millipore) was used throughout the experiment.

CNTs were purified using ultrasonic treatment in 32 wt % HCl solution (Ajax Finechem) for 6 h and then stirred at room temperature for 48 h. CNTs were characterized by TEM (JEOL 3000F) with an operation voltage of 200 kV, and the BET surface area of CNTs was measured by Micromeritics TriStar II. PBS solution (0.1 M, pH 7.4) were prepared by mixing 0.1 M NaH2PO4 and 0.1 M Na2HPO4, and the pH of the solution was adjusted by adding H3PO4 or NaOH. Glucose stock solution was prepared in the present study, the use of CNT supports with 2 inner tubes would significantly enhance the activity and sensitivity of the electrocatalysts in applications such as biofuel cells and glucose biosensors.

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4.2. Preparation of GOX/CNT-Modified Electrodes. The 4.0 mm diameter GC electrode was polished with 3 μm alumina powder and sonicated in deionized water for 5 min, and it served as the working electrode. After drying, the GOX/CNT film electrodes were prepared by successively casting 5 μL of CNT suspensions and GOX solution on the electrode surface. After CNTs and GOX solution deposition, the solvent was allowed to evaporate at room temperature. To protect the working electrode, 5 μL of Nafion solution was dropped on the surface of the cast electrode films and dried at room temperature.

4.3. Characterization. Electrochemical measurements were performed in a conventional three-electrode cell with an Ag/AgCl reference electrode and a Pt counter electrode in N2- or O2-saturated 0.1 M PBS solution, using a CHI6044D electrochemical work station. The Ag/AgCl reference electrode was kept in saturated KCl solution. CVs of the bare GC and GOX/CNT-modified GC electrodes were generally obtained at a scan rate of 50 mV s⁻¹. The amperometric measurements were performed at a fixed potential (~0.48 V vs Ag/AgCl). Glucose (100 μL, 0.1 M) was directly added to 40 mL of 0.1 M PBS solution at 0 s with the successive increase of the glucose concentration (100 μL, 0.1 M glucose). The reason for performing the amperometric measurements at this fixed potential is to maximize the activity and sensitivity of measurements, as ~0.48 V is close to the cathodic peak potential of direct electrochemistry of GOX/CNT-modified GC, as shown in the results section. The experiments were repeated at least three times, and the results were presented as the average values.

4. EXPERIMENTAL SECTION

4.1. Materials and Solutions. Glucose oxidase (GOX, EC 1.1.3.4, 100–250 U/mg, type X-S from Aspergillus niger), D-(-)-glucose, DMF, sodium phosphate monobasic, and sodium phosphate dibasic were purchased from Sigma Aldrich. Nafion solution (5 wt % in isopropanol and water) was purchased from DuPont Inc. Four CNTs with different number of walls were purchased from Nanostructured & Amorphous Materials Inc., USA. Deionized double-distilled water (18.6 MΩ, Millipore) was used throughout the experiment.

CNTs were purified using ultrasonic treatment in 32 wt % HCl solution (Ajax Finechem) for 6 h and then stirred at room temperature for 48 h. CNTs were characterized by TEM (JEOL 3000F) with an operation voltage of 200 kV, and the BET surface area of CNTs was measured by Micromeritics TriStar II. PBS solution (0.1 M, pH 7.4) were prepared by mixing 0.1 M NaH2PO4 and 0.1 M Na2HPO4, and the pH of the solution was adjusted by adding H3PO4 or NaOH. Glucose stock solution was prepared by PBS solution and stored for at least 24 h at room temperature before use. CNTs (~2 mg) were dispersed into 5 mL DMF and then ultrasonicated for 1 h to form a stable CNT suspension. GOX solution (10 mg mL⁻¹) was stored in −4 °C. All aqueous solutions were prepared with double-distilled deionized water.

4.2. Preparation of GOX/CNT-Modified Electrodes. The 4.0 mm diameter GC electrode was polished with 3 μm alumina powder and sonicated in deionized water for 5 min, and it served as the working electrode. After drying, the GOX/CNT film electrodes were prepared by successively casting 5 μL of CNT suspensions and GOX solution on the electrode surface. After CNTs and GOX solution deposition, the solvent was allowed to evaporate at room temperature. To protect the working electrode, 5 μL of Nafion solution was dropped on the surface of the cast electrode films and dried at room temperature.

4.3. Characterization. Electrochemical measurements were performed in a conventional three-electrode cell with an Ag/AgCl reference electrode and a Pt counter electrode in N2- or O2-saturated 0.1 M PBS solution, using a CHI6044D electrochemical work station. The Ag/AgCl reference electrode was kept in saturated KCl solution. CVs of the bare GC and GOX/CNT-modified GC electrodes were generally obtained at a scan rate of 50 mV s⁻¹. The amperometric measurements were performed at a fixed potential (~0.48 V vs Ag/AgCl). Glucose (100 μL, 0.1 M) was directly added to 40 mL of 0.1 M PBS solution at 0 s with the successive increase of the glucose concentration (100 μL, 0.1 M glucose). The reason for performing the amperometric measurements at this fixed potential is to maximize the activity and sensitivity of measurements, as ~0.48 V is close to the cathodic peak potential of direct electrochemistry of GOX/CNT-modified GC, as shown in the results section. The experiments were repeated at least three times, and the results were presented as the average values.

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