

Effect of Surface-oxidized Structure of Single-walled Carbon Nanotubes on Heterogeneous Direct Electron-transfer Reaction of Cytochrome *c*

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The redox reaction of cytochrome *c* was not observed at as-grown, high-quality, single-walled carbon nanotubes (SWCNTs) synthesized onto a gold electrode surface. On the other hand, at a gold electrode modified with SWCNTs with oxidized carbon species such as C–O, C=O, and O–C=O induced by UV–ozone treatment, a well-defined diffusion-controlled redox wave of cytochrome *c* was observed. The results clearly indicated that oxidized carbon species such as C–O, C=O, and O–C=O play an important role for the fast heterogeneous direct electron-transfer reaction of cytochrome *c* with CNTs.

CNTs have been widely used as a platform electrode material for the preparation of enzyme sensors, because of their unique properties in fundamental studies as atomically ordered materials, their large surface area-to-volume ratios, and their potential in nanotechnological applications.^{1–3} It is expected that CNTs can function as nanowires for electron-transfer communication with the redox center of an enzyme because of their small diameters and conductivities.^{4,5} However, the relationship between the oxidized carbon species derived from the surface defects of the CNTs and the electron-transfer reaction with an enzyme has not been investigated. In this study, we focused on the effect of the oxidized carbon species derived from the surface defects of CNTs on protein electrochemistry. We directly synthesized single-walled carbon nanotubes on a gold electrode surface (SWCNT/Au) to obtain as-grown, high-quality CNTs and investigated the redox reaction of cytochrome *c* as a standard redox protein. Fast electron-transfer reaction of cytochrome *c* has been reported at an oxidized-SWCNT-modified glassy carbon electrode.⁶ However, such oxidation effect of CNTs on the electrode reaction of cytochrome *c* has not been investigated. Our obtained results clearly indicated that the redox reaction of cytochrome *c* was strongly influenced by the presence of the oxidized carbon species on the SWCNT surface.

Cytochrome *c* (from horse heart, Sigma) was used as received. The concentration of cytochrome *c* was estimated spectroscopically by using a reduced-form molar absorptivity coefficient of $29500 \text{ M}^{-1} \text{ cm}^{-1}$ at 550 nm .⁷ All other reagents used in this study were of analytical grade.

Cyclic voltammetric measurements were performed in a phosphate buffer (pH 7) solution. An Ag/AgCl (saturated KCl) electrode and a platinum electrode were used as the reference and counter electrodes, respectively. All potentials were reported with respect to the Ag/AgCl (saturated KCl) electrode. X-ray photoelectron spectroscopic (XPS) measurements were performed using a Thermo VG Scientific Sigma Probe HA6000II. This instrument uses a focused monochromatic AlK α X-ray (1486.68 eV) source for excitation. The binding energies were calculated on the basis of a binding energy of C(1s) (284.5 eV). A UV–ozone treatment system (Model OC-2503, Eye Graphics Co., Japan) was used in this study. The UV light had main emissions at 185 and 254 nm. The density at 230–280 nm (sensitivity peak: ca. 255 nm) was evaluated as 11 mW cm^{-2} by an ultraviolet ray integration luminance meter (UVPF-A1, Eye Graphics Co., Japan).⁸

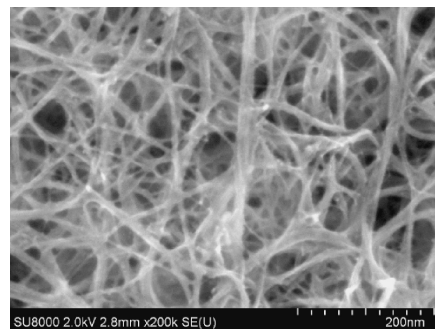


Figure 1. FE-SEM image of the SWCNT-modified Au electrode surface.

The SWCNTs were synthesized onto a gold wire using Co–Mo alloy nanoparticles as the catalyst.² The CVD growth of the SWCNTs was carried out in a quartz tube equipped with temperature and gas flow controls. The prepared catalyst nanoparticles on the electrodes were first reduced in H_2 for 10 min at $750 \text{ }^\circ\text{C}$, and then a mixture of ethanol (99.8%) and H_2 gas was introduced into the system at a flow rate of 100 mL min^{-1} H_2 at $750 \text{ }^\circ\text{C}$ for 10 min. The synthesized CNTs were characterized by Raman spectroscopy (JASCO, NRS-3100), FE-SEM (HITACHI, SU-8000), and TEM (JEOL, 2000FX). Figure 1 shows the FE-SEM results at a gold electrode surface modified with CNTs. This clearly showed that bundles of CNTs (bundled size: 5–20 nm in diameter) were uniformly distributed on the electrode surface. From the TEM image, individual CNTs were observed at the sample of Figure 1, as shown in Figure 2. The diameter was evaluated to be 1.0–1.5 nm, and they were, therefore, classified as mainly single-walled CNTs (SWCNTs). Two Raman shift peaks corresponding to the G- (ca. 1593 cm^{-1}) and D-bands (ca. 1350 cm^{-1}) were observed from SWCNTs on the gold wire electrode surface. The ratios of the G-band and D-band (G/D) were evaluated to be 20 or less for the as-grown SWCNTs when a laser at 532 nm was used for excitation. The results from the radial breathing mode (RBM) of the Raman spectroscopic measurements indicated that the SWCNTs ranged in diameter between 1.2–1.5 nm,⁹ which was the same size value obtained from the TEM results.

To induce the oxidized carbon species derived from surface structural defects on the SWCNT surface,¹⁰ UV–ozone treatment was performed for 1–5 min. The SEM and TEM images of UV–ozone-treated SWCNTs for 5 min were almost the same as the untreated SWCNTs. The difference in the images was not clear. On the other hand, decreasing G-band intensity and increasing D-band intensity were observed with the UV–ozone treatment time, and thus the G/D ratio gradually decreased with the UV–ozone treatment. The values of G/D ratio were 8, 5, 4, and 3 for 0.5, 1, 2, and 5 min, respectively. The XPS results of the UV–ozone-treated SWCNT/Au electrode in the C(1s) region showed peaks corresponding to a C–C bond (ca. 285 eV), and other oxidized

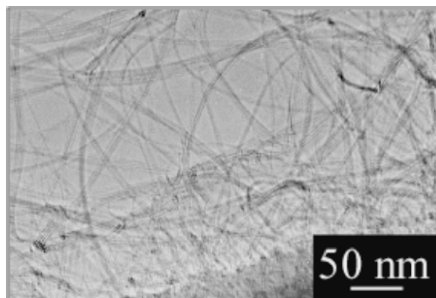


Figure 2. TEM image of the SWCNT on Au electrode surface.

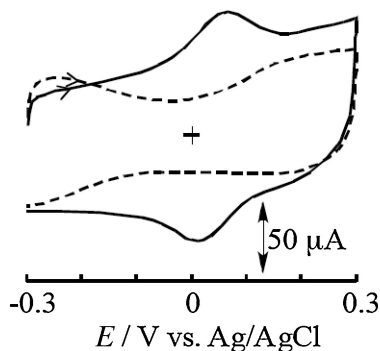


Figure 3. Cyclic voltammograms of UV-ozone-treated (solid line) and untreated (broken line) SWCNT/Au electrode in a phosphate buffer (pH 7) solution of 0.1 mM cytochrome *c*. Potential sweep rate: 80 mV s⁻¹. Electrode area: 0.25 cm².

carbon species such as C–O, C=O, and O–C=O (ca. 286–290 eV) were observed.¹¹ The peak area ratios of the oxidized carbon species to the total carbon species were evaluated to be ca. 10, 20–30, and ca. 60% for UV-ozone exposures of 0, 1, and 5 min, respectively. These results indicated that surface structural defects on the SWCNTs were caused by the UV-ozone treatment.

Figure 3 shows typical cyclic voltammograms at the SWCNT/Au electrode in a cytochrome *c* solution. No redox wave of cytochrome *c* was observed at the electrode modified with the as-grown SWCNTs. On the other hand, a well-defined redox wave of cytochrome *c* was observed at the UV-ozone-treated SWCNT/Au electrode for 5 min. The results at the UV-ozone-treated SWCNT/Au electrode for 2, 3, and 5 min were similar to each other. The peak current was proportional to the square root of the potential sweep rate at ca. 300 mV s⁻¹, which indicated that the redox reaction was a diffusion-controlled process and was not due to the adsorption species of cytochrome *c*. The redox potential of cytochrome *c* was evaluated to be 35–60 mV (vs. Ag/AgCl/saturated KCl), which was almost the same value as that evaluated at a promoter-modified gold electrode and indium oxide electrode.^{12,13} These results clearly indicated that the cytochrome *c* molecule retained the same native structure during the redox reaction. The well-defined redox wave of cytochrome *c* was only observed at noncontaminated SWCNTs with UV-ozone treatment. The redox reaction of cytochrome *c* was immediately inhibited, when the treated SWCNT-modified Au electrode was exposed to an atmosphere.

These results clearly indicated that the presence of oxidized carbon species such as C–O, C=O, and O–C=O due to the surface structural defects of the SWCNTs play an important role in the fast electron-transfer reaction of cytochrome *c* with native structure.

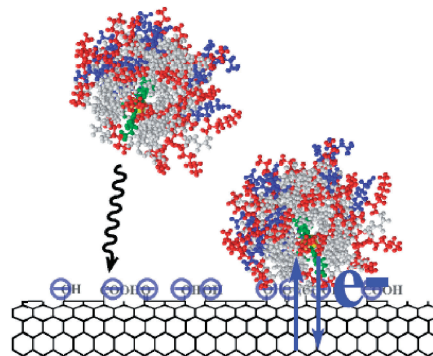


Figure 4. Schematic illustration for electrostatic interaction of cytochrome *c* and SWCNT surface for direct electron-transfer reaction.

Cytochrome *c* has positively charged lysine residue around the heme crevice. The graphene sheet of carbon nanotube has negatively charged functional group such as a carboxylic acid after the UV-ozone treatment. It is well known that molecular orientation of cytochrome *c* on an electrode surface is important for fast heterogeneous electron-transfer reaction with the electrode.¹³ Electrostatic interaction between positively charged lysine residue of cytochrome *c* and negatively charged functional groups of SWCNTs would occur as shown in Figure 4, resulting in that cytochrome *c* molecule would orient on the SWCNT surface for the electron-transfer reaction.

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