

Catalytic Current Based on Direct Electron Transfer Reactions of Enzymes Immobilized onto Carbon Nanotubes

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Direct heterogeneous electron transfer reactions between proteins (enzymes) and electrodes have been extensively studied to understand the fundamental features of proteins, and for applications such as biodevices, biosensors and enzyme-biofuel cells. In general, the electron transfer rate of an enzyme on an electrode surface is slow, or cannot be detected due to the fact that the redox center of the enzyme is deeply buried within the protein shell. Carbon nanotubes could be communicate with redox centers buried deeply within protein shells as molecular wires because of their small diameters and conductivities. Herein, we report for the investigation of heterogeneous direct electron transfer reactions of enzymes immobilized onto carbon nanotubes.

Glucose oxidase (GOX, EC 1.1.3.4, from *Aspergillus niger*, 12 units mg^{-1} , Tokyo Chemical Ind. Co., Japan) and D-fructose dehydrogenase (FDH, EC 1.1.99.11, from *Gluconobacter* sp., Toyobo Co., Japan) were used as received. The electrochemical measurements were carried out in a three-electrode cell. Cyclic voltammetric measurements were performed in a phosphate buffer (pH 7) and phosphate (pH 5) solutions using an electrochemical analyzer. An Ag/AgCl (saturated KCl) electrode and a platinum electrode were used as the reference and counter electrodes, respectively. All potentials are reported with respect to the Ag/AgCl (saturated KCl) electrode. Prior to the cyclic voltammetric measurements, the buffer solution was deaerated with high purity argon, and a positive pressure of argon was kept over the solution during electrochemical experiments.

Carbon nanotubes (CNTs) was synthesized on a platinum plate (20×20×1 mm) by the chemical vapor deposition method using iron nanoparticles derived from ferritin molecules [1]. The synthesized CNTs were characterized by Raman spectroscopy and TEM. Fig. 1 shows a typical TEM image of CNTs. The diameters of individual CNTs were evaluated to be *ca.* 5 to 10 nm, which could be classified as multi-walled CNTs (MWCNTs). Immediately thereafter, the MWCNTs on the Pt plate (MWCNTs/Pt) electrode were immobilized into enzyme.

First, we investigated the effect of the iron nanoparticles on the electrochemical response. Before the MWCNT synthesis, XPS peaks around 712 and 725 eV corresponding to Fe(2p) were observed. However, these Fe(2p) peaks could not be detected after the MWCNT synthesis. After the elimination of MWCNT by heat-treatment at 900 °C, the peaks corresponding to Fe(2p) were observed again. These facts indicated that iron nanoparticles were present on the electrode surface, but the nanoparticle surface was enclosed with MWCNTs. The cyclic voltammetric investigation also supports such a hypothesis. A peak based on iron impurity species could not be detected on the MWCNTs/Pt electrode in a phosphate buffer (pH 7) solution, indicating that the iron

nanoparticle surfaces were electrochemically inactive.

Fig. 2a shows typical cyclic voltammograms at GOX immobilized onto the MWCNTs/Pt electrode in the presence and absence of glucose. The well-defined catalytic oxidation current was observed from *ca.* -0.45 V. In contrast, no catalytic current was observed at either Pt or MWCNTs/Pt electrodes. The potential that catalytic current increased was close to the redox potential of flavin adenine dinucleotide (FAD) as a prosthetic group of GOX under physiological pH values. Thus, the catalytic oxidation current would be due to the direct electron transfer reaction of GOX and the MWCNTs/Pt electrode.

Fig. 2b shows typical cyclic voltammograms at FDH immobilized MWCNTs/Pt electrodes in the presence and absence of fructose. No catalytic current was observed at either Pt or MWCNTs/Pt electrodes in the potential region of -0.2 to 0.6 V in the presence of fructose. In contrast, a well-defined catalytic oxidation current was observed from *ca.* -0.15 V at the FDH immobilized MWCNTs/Pt electrode, which was close to the redox potential of heme *c* as a prosthetic group of FDH. In our previous report, direct heterogeneous electron transfer reactions of FDH adsorbed onto a HOPG electrode were also observed from about -0.15 V [2]. Thus, in the case of FDH immobilized onto MWCNTs/Pt electrode, the electron transfer reactions would pass between the MWCNTs/Pt electrode and FDH, which was similar to the case of FDH adsorbed onto HOPG.

The obtained results clearly indicate that carbon nanotubes are useful for applications to prepare the third-generation biosensors and other future bioelectrochemical devices.

REFERENCES

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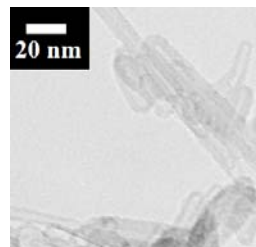


Fig. 1 TEM image of carbon nanotubes synthesized on an electrode surface.

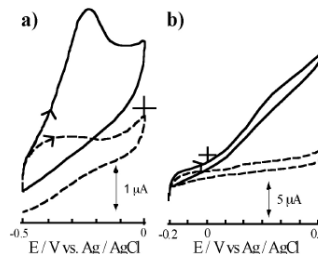


Fig. 2 Typical cyclic voltammograms at GOX (a) and FDH (b) immobilized MWCNTs/Pt electrodes in a buffer solution in the presence (solid lines) and absence (broken lines) of substrates.