

## Electrochemical behaviors of fructose dehydrogenase immobilized onto UV-ozone-treated carbon nanotubes modified electrode

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### Abstract

Carbon nanotubes (CNTs) were synthesized onto a gold electrode. Fructose dehydrogenase (FDH) was immobilized onto the UV-ozone-treated CNTs on gold electrode (UV-CNT/Au). Catalytic oxidation currents based on the direct electron transfer reactions of FDH were observed at FDH modified UV-CNT/Au electrode. The observed catalytic oxidation currents strongly depended on the UV-ozone treatment time.

### Introduction

The increasing interest in direct electron transfer (DET) reaction type enzyme is driven by its important applications as biosensors, biofuel cells and bioreactors. CNTs are used as an electrode for DET reactions with enzymes, because CNTs have a high conductivity and small diameter. However, the influence of surface conditions of CNTs on the DET reactions with enzymes has not been investigated. In this study, we found, for the first time, that the DET reaction of enzymes at CNT surface was strongly influenced with the surface structural defects of CNTs.

### Experimental

CNTs were synthesized onto a gold electrode surface by using chemical vapor deposition method using Co-Mo alloy nanoparticles as a catalyst. From Raman spectroscopic results and TEM image (Fig. 1) the diameters of synthesized CNTs were evaluated to be ca. 1~1.5 nm. To induce a surface structural defect onto the CNTs, UV-ozone treatment was performed for 1 and 5 min. D-fructose dehydrogenase (FDH, EC 1.1.99.11) was purchased from Toyobo Co., Japan, and used without further purification. FDH is consisted from three subunits including flavin and heme c as prosthetic group. To immobilize FDH onto the CNT surface, the CNTs modified gold (CNT/Au) electrode was immersed into phosphate solution (pH 5) of 1 unit  $\mu\text{l}^{-1}$  FDH for 1 min.

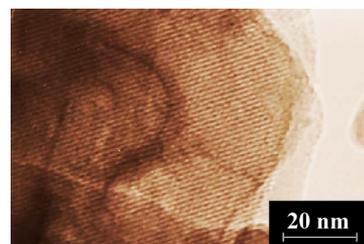


Fig. 1 TEM image for CNTs grown on a gold electrode.

## Results and Discussion

Fig. 2 shows the X-ray photoelectron spectroscopic (XPS) results at the UV-ozone-treated CNT/Au (UV-CNT/Au) electrode in the C(1s) region. Ratio of peaks corresponding to the oxidized carbon species such as C-O, C=O and O-C=O (ca. 286-290 eV) were increased with UV-ozone treatment time. These results indicated that the surface structural defects of CNTs were induced by UV-ozone treatment. Fig. 3 show typical cyclic voltammograms at FDH-modified and -unmodified CNT/Au electrodes. Catalytic oxidation currents were observed from ca. -0.1 V in a phosphate solution (pH 5) in the presence of 0.1 M fructose. On the other hand, no catalytic oxidation current was observed at the unmodified CNT/Au electrode. These results indicate that the observed catalytic oxidation current based on the DET reactions of FDH immobilized onto the CNT/Au electrode. The UV-ozone-treated CNT for 1 min was most suitable electrode for DET reaction with FDH in comparison with other electrodes. To clarify the reason, we evaluated the surface excess of FDH on the CNT/Au electrode by using fluorescent measurement. The surface excess of FDH was evaluated to be  $4.5 (\pm 1.0) \times 10^{-11} \text{ mol cm}^{-2}$ , which did not depend on the UV-ozone treatment time. These results indicate that the difference in the catalytic current values was not due to the surface excess of FDH. It is well known that the molecular orientation of the enzyme on the electrode surface is one of the most important requirements for fast DET reactions, because the redox center of the enzyme is buried deeply within the protein shell. The proper orientation of FDH on an electrode surface is also important for successful its DET reactions [1]. From the fact that the catalytic oxidation current based on the DET reaction of FDH increased from ca. -0.1 V, the DET reaction of FDH at the electrode surface occurred at the heme c-containing subunit [1]. From the result of the observation of catalytic oxidation from -0.1 V together with the fact that surface excess of FDH did not depend on UV-ozone treatment time, the difference in the observed catalytic current values at the UV-CNT/Au electrodes was came from the difference in the proper orientation of FDH on the CNT surface.

1 M. Tominaga, C. Shirakihara, and I. Taniguchi, *J. Electroanal. Chem.*, **610**, 1-8 (2007).

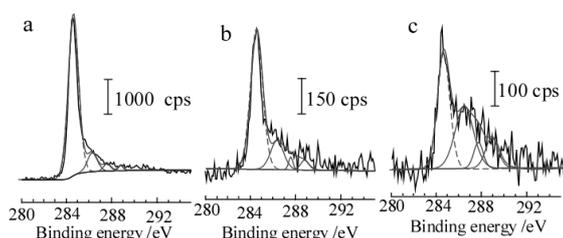


Fig. 2 XPS results in the C(1s) region at CNT/Au electrodes treated with UV-ozone for 0 (a), 1 (b) and 5 min (c).

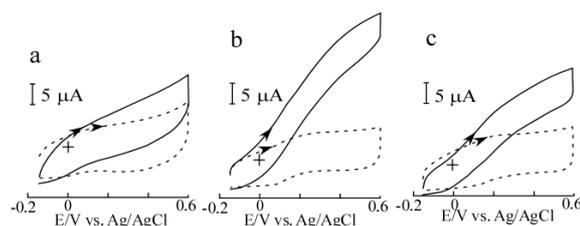


Fig. 3 Cyclic voltammograms at the UV-ozone treated (a: 0, b: 1 and c: 5 min) CNT/Au electrodes modified with FDH in a phosphate solution (pH 5) in the presence (solid line) and the absence (broken line) of 0.1 M fructose. Potential sweep rate:  $5 \text{ mV s}^{-1}$ . Electrode area:  $0.25 \text{ cm}^2$ .