

Functional Modified Au Electrodes for Direct Electron-Transfer (DET) Reaction of Fructose Dehydrogenase (FDH) and Their Application to Bio-fuel Cells

Chiharu Shirakihara,

Masato Tominaga, Katsuhiko Nishiyama,
Isao Taniguchi

Department of Applied Chemistry and
Biochemistry, Kumamoto University
2-39-1, Kurokami, Kumamoto, 860-8555,
Japan

Email: taniguch@gpo.kumamoto-u.ac.jp

Direct electron-transfer (DET) type bioelectrocatalysis has been received much attention in recent years in its application to biosensors, biofuel cells and bioreactors.

D-fructose dehydrogenase (FDH) is a membrane-bound enzyme with a molecular weight of ca. 140 kDa containing flavin and heme c as prosthetic groups. This enzyme shows high substrate specificity to D-fructose, which is expected to be applied in the field of food analysis and clinical use. In the present paper, direct heterogeneous electron transfer reactions and the molecular orientation of FDH adsorbed on the electrode surfaces will be discussed for potential application to bioelectrochemical devices.

FDH (EC 1.1.99.11, from *Gluconobacter sp.*) was purchased from Toyobo Co., Japan, and was used without further purification. SAMs of thiol molecules shown in Fig. 1 were used. SAM modified Au electrodes were prepared by immersion of the electrodes for 20 min into a 0.1 mmol dm⁻³ solution of a given modifier. FDH was then immobilized on the SAM modified Au electrodes by immersion of the electrodes for 1 min into a 0.1 mmol dm⁻³ phosphate buffer solution (pH 5.0) of 1 unit μl⁻¹ FDH.

Cyclic voltammetric and differential pulse voltammetric measurements were performed using an Ag/AgCl (saturated KCl) electrode and a Pt plate as the reference and auxiliary electrodes, respectively.

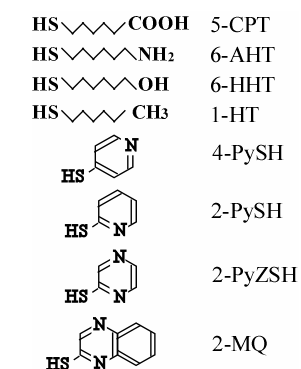


Fig. 1 Surface modifiers used 5-CPT, 6-AHT, 6-HHT, 1-HT, 4-PySH, 2-PySH, 2-PyZSH, 2-MQ.

FDH is consisting of three subunits including flavin and heme c as active centers. Catalytic oxidation currents based on the DET reactions of FDH adsorbed on the Au electrodes were observed around -0.1 V (vs. Ag/AgCl/saturated KCl) in a phosphate buffer solution (pH 5.0) in the presence of 0.1 M fructose. On 5-CPT, 6-AHT, 6-HHT, 1-HT modified Au electrodes, no catalytic oxidation current based on the DET reactions of FDH was observed other than 5-CPT on Au electrode. It was suggested that a negative charge of the electrode surface would be important for the proper orientation of FDH [1].

On the other hand, when 4-PySH, 2-PySH, 2-PyZSH were used as modifiers of Au electrodes from the viewpoint of comparison with DET reactions of cytochrome c on the functional electrodes, catalytic oxidation currents based on the DET reactions of FDH was observed on the electrodes on which DET of cytochrome c was observed (4-PySH, 2-PyZSH, 2-MQ modified Au electrodes. These results may indicate the protein surfaces of both FDH and cytochrome c would be similar to each other.

By using FDH adsorbed Au nano-particles modified carbon felt electrodes as an anode for oxidation of D-fructose and laccase adsorbed carbon-felt electrode as a cathode for oxygen reduction, and an interesting DET-type bio-fuel cell can be prepared.

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

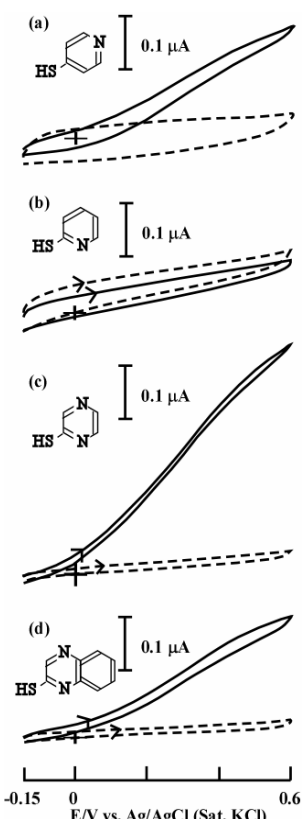


Fig. 2 CVs of fructose oxidation at FDH immobilized 4-PySH, 2-PySH, 2-PyZSH, 2-MQ modified Au(111) electrodes ((a), (b), (c), (d)) in the presence (solid line) and absence (broken line) of 0.1 M fructose. (Scan rate: 5 mV/s)