In Situ Observation of Electron Transfer Reaction of Surface Immobilized Cytochrome _c_ on ITO Electrode Using Slab Optical Waveguide Spectroscopy

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It is essential to obtain *in situ* information of the molecules adsorbed on electrodes for understanding and controlling their electrochemical reactions. However, it is difficult to observe the absorption spectra of molecules adsorbed on electrodes because of the lack of sensitivity with conventional techniques. Recently we have developed a novel technique using slab optical waveguide (SOWG) spectroscopy, with which absorption spectra of adsorbed materials were observed. Additionally, we formed indium-tin-oxide (ITO) electrode thin film on a SOWG (ITO-SOWG) for *in situ* observation of molecules adsorbed on electrode surface.[1]

The SOWG system was similar to that described previously.[1-3] A 50-μm thick glass plate was used as a SOWG and the thickness of ITO films were about 20 nm. The cell length was about 1.5 cm and the surface area of ITO-SOWG covered with sample solution was about 3 cm^2. The guided light was propagated through the glycerol drop on the SOWG. The electrode potential was controlled with a potentiostat (PAR Model 273A). The counter and reference electrodes were Pt wire and Ag/AgCl, respectively. Cytochrome *c* was purchased from Kanto Chemical Co., Inc. and used as received.

In this study, the electron transfer reactions of surface immobilized cytochrome *c* were investigated by in situ observation of absorption spectra by ITO-SOWG spectroscopy. The absorption spectral change of cytochrome c obtained by SOWG spectroscopy were shown in Figure 1. The redox potential of surface immobilized cytochrome *c* was estimated to be about –0.2 V vs. Ag/AgCl from the absorbance intensity change with respect to applied potential scan to ITO electrode. These results suggested that after adsorption on ITO electrode, cytochrome *c* was still electrochemically active. Additionally direct electron transfer between surface immobilized cytochrome *c* and ITO electrode without supporting electrolyte was investigated. The peak position of the absorption band of cytochrome *c* immobilized on ITO electrode without supporting electrolyte changed repeatedly between 409 and 414 nm with the electrode potential sweep, which corresponded to the oxidized and reduced form of cytochrome *c*, respectively. The response time of surface immobilized cytochrome *c* was observed by time resolved absorption spectra. These results showed that surface immobilized cytochrome *c* on ITO electrode was still electrochemically active. Futhermore, our system is so highly sensitive that it is possible to obtain *in situ* absorption spectra from under monolayer coverage and with short time duration as time-resolved spectra.

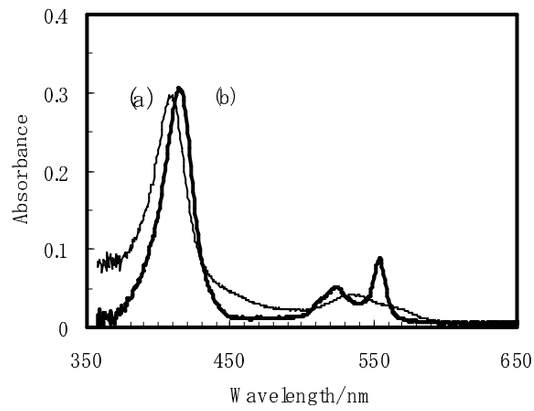

Figure 1. Absorption spectra of surface immobilized cytochrome *c* obtained by slab optical waveguide spectroscopy utilizing indium-tin-oxide electrode. The electrode potential was set at 0 and -0.5 V vs Ag/AgCl in Fig. 2(a) and (b), respectively. The electrolyte solution was phosphate buffer solution (pH=6.9).

References : [1] Naoki Matsuda et al., *Chem. Lett*., **1998**, 125 (1998). [2] Naoki Matsuda et.al., *Thin Solid Films*, **438-439**, 403 (2003). [3] Naoki Matsuda et.al., *Thin Solid Films*, **445**, 313 (2003).