

Non-contact Optical Waveguide Spectroscopy: in-situ spectral analysis of cytochrome *c* immobilized on mixed SAM modified gold electrode

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Introduction

The electrochemical reaction of proteins existed on the electrode has generally been analyzed by CV measurements. On the other hand, spectroelectrochemistry gives useful information such as electronic state of protein active center. However, spectral analysis of opaque electrodes like gold and carbon is impossible by ordinary transmission UV-vis spectroscopy. To enable the spectral analysis of molecules on the substrate regardless of its optical transparency, we proposed non-contact optical waveguide (NOW) spectroscopy^{1,2}. In this study, the spectral analysis of the redox reaction of proteins on the opaque electrode has been carried out by a combination of NOW spectroscopy and CV measurement.

Experimental

L-shaped gold electrode was cleaned by reported procedure. To modify self assembled monolayer (SAM) on the gold surface, cleaned gold electrode was soaked in mixed ethanol solution of HOOC(CH₂)SH:HO(CH₂)₆SH=2:1 overnight. After that, the electrode was incubated in an aqueous solution of EDC (200mM) and NHS (50mM) for 10 min. The electrode was rinsed with buffer and incubated in 50 μ M cyt.*c* solution overnight to fix cyt.*c* covalently on the mixed SAM modified gold surface. The electrode was then sonicated in 1M phosphate buffer to remove non-covalently adsorbed cyt.*c* and rinsed with the buffer before NOW measurements. The NOW measurements were carried out by using the latex beads (d: 120nm) as a spacer. Redox reaction of cyt.*c* fixed on the mixed SAM modified gold electrodes was observed by using the electrochemical cell system constructed on the waveguide. Pt and Ag wires were used as a counter and a reference electrode, respectively. The potential was applied by a potentiogalvanostat. The current change observed in this cell system was recorded at the same time.

Results and Discussion

NOW spectroscopy certainly detected the absorption spectra of cyt.*c* covalently fixed on the mixed SAM modified gold electrode. The Soret band was observed at 408 nm which was the same as that of cyt.*c* solution observed by ordinary UV-vis spectroscopy. With potential sweep to negative direction, the spectrum at the Soret band showed a red shift from 408 nm to 414 nm due to the reduction of heme. The λ_{max} of Soret band returned to 408 nm by the re-oxidation. The redox response was observed repeatedly by the potential sweep (Fig.1). When the sweep rate was changed from 10 to 300 mV/sec, absorbance showed the corresponding response to the sweep rate. It was suggested that electron transfer was fast enough to follow the potential sweep in this range. Cyclic voltammogram of this reaction was observed simultaneously by electrochemical cell system constructed on the waveguide. Observed cyclic voltammogram showed the proportional relationship

between the potential sweep rate and the peak currents. It exhibits that the electron transfer process was the rate-determining step of this reaction.

Differential plot of the absorbance change was plotted against the applied potential to determine the redox potential (Fig.2 (A)). The redox potential obtained by changes in NOW spectra agreed with that deduced by ordinary CV.

References

- 1) K.Fukuda and H.Ohno, *Electroanalysis*, 14, No.9, 605-610 (2002)
- 2) K.Fujita, C.Suzuki, H.Ohno, *Electrochem. Commun.* 5, 47-50 (2003)

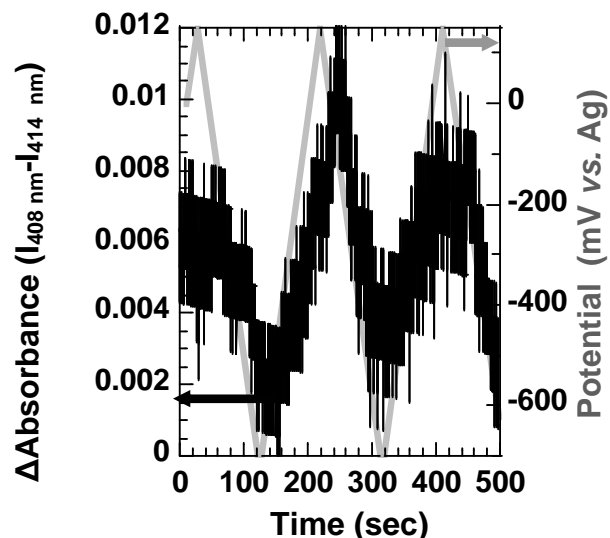


Fig.1 Time dependence of Δ Absorbance ($I_{408nm} - I_{414nm}$) for cyt.*c* covalently bound to mixed SAM modified gold electrode (10mV/sec).

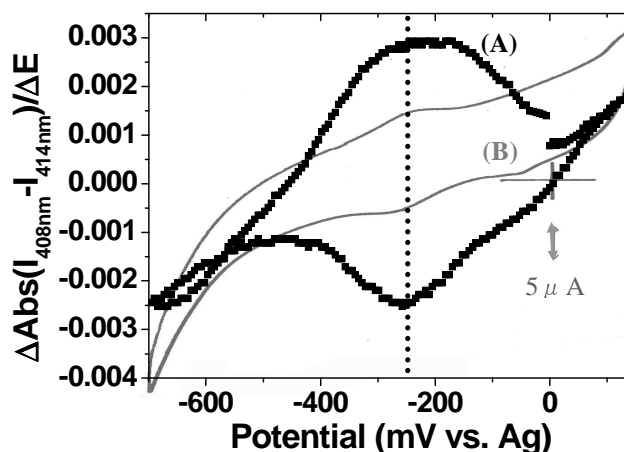


Fig.2 (A) Differential plot of Δ Absorbance ($I_{408nm} - I_{414nm}$) (B) Cyclic voltammogram detected by electrochemical cell on the waveguide. (50mV/sec)