

Electron-Transfer Reactions and Structural Properties of Heme Proteins Immobilized on Electrodes

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Monolayers of proteins on electrodes are interesting for the investigation of intermolecular electron transfer as well as for the development of bioelectronic devices. In this research, three heme proteins (myoglobin; Mb, cytochrome *c*; Cyt *c*, and cytochrome *c*₅₅₁; Cyt *c*₅₅₁) were immobilized on the electrodes and studied using electrochemical methods and surface-enhanced resonance Raman spectroscopy (SERRS). A mixed monolayer film of 1-octanethiol and 1-(11-mercaptoundecyl)imidazole [1] has been used to adsorb Mb to the surface of a gold electrode. Cyt *c* and Cyt *c*₅₅₁ have been immobilized on modified electrodes with phenylglyoxal (PG) derivatives which react selectively with a guanidino group of arginine.

Mb immobilized on the mixed SAM provided an apparent redox response, although there was no redox response at the SAM which was consisted of only 1-octanethiol. It suggests that the imidazole moiety of the SAM contributes the immobilization and redox reaction of Mb. A potential of the response was estimated ca. -460 mV *vs.* Ag / AgCl. A large negative-shift in the redox potential also indicates that the sixth position of the heme iron is occupied by a nitrogen atom of the imidazole moiety. To investigate coordination configuration of heme, the SERRS measurements of Mb immobilized on the mixed SAM electrode were performed. The ν_2 and ν_3 bands in resonance Raman spectra of heme proteins sensitively exhibit the spin state of the heme iron. The ν_2 and ν_3 bands of Mb on the mixed SAM were observed at 1583 and 1503 cm⁻¹, respectively. Since the values are the almost same as those of the Mb-imidazole complex (6cLS), it is strongly confirmed that the imidazole moiety binds to the redox center of Mb on the electrode surface.

Cyt *c* and Cyt *c*₅₅₁ immobilized on a *p*-glyoxyloylphenyl 3-mercaptopropionate (GPMP) modified gold electrode showed redox responses and the potentials were +136 mV and +164 mV, respectively. These results indicate that the GPMP-SAMs on the electrodes could bind guanidino groups of Arg residues on the protein surfaces. The structures of cytochromes on the PG derivatives would be reported on the basis of the SERRS measurements.

[1] J. Wei et al., *J. Am. Chem. Soc.*, **124**, 9591-9599 (2002).