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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Biological electron transfer, which is high efficient electron transfer observed in the electron transfer chain such as respiration or photosynthesis, was controlled by each redox protein. Redox protein selects a redox partner and maintains the electron transfer chain. Biological electron transfer is consisted of intramolecular electron transfer, which means an electron transfer within a single protein, and intermolecular electron transfer, which means electron transfer between redox protein and redox partner.

Then, there are unknown a lot of electron transfer mechanisms yet, since the electron transfer reactions arising from redox protein are complicated.

Cytochrome c_3 from *Desulfovibrio vulgaris* (Miyazaki F), a multi redox protein, contains four hemes which were coordinated bis-histidine. In biological processes, cytochrome c_3 accepts electron from hydrogenase in intramolecular electron transfer and transports electron to cytochrome *c* for cellular respiration. Cytochrome c_3 has multiple redox state arising from conformational change during intramolecular electron transfer among hemes to occur intermolecular electron transfer with hydrogenase.

In this study, protein film voltammetry (PFV) was carried out to investigate how intramolecular electron transfer occurs within cytochrome c_3. PFV, which is a direct electrochemical measurement allowing direct observation of electron transfer of a redox protein.

The idea behind PFV, which is a method of direct electrochemistry, is that the protein molecules under investigation are immobilized on an electrode surface and redox centers undergo interfacial electron exchange. PFV is able to directly investigate electron transfer in redox protein by electrochemical technique such as voltammetry.

PFV experiments were carried out using a three-electrode configuration. A platinum electrode was used as a counter electrode and a silver/silver chloride (Ag/AgCl) electrode (+0.199 V *vs.* SHE) was used as a reference electrode. Protein films were prepared on pyrolytic graphite edge (PGE), 5-(1,2-dithiolan-3-yl)pentanoic acid (lipoic acid)-modified gold, and cystamine sulfate-modified gold electrodes. 10 mM mixed buffer of MES, HEPES, and TAPS at pH 7.0 was used.

Direct electrochemical measurement shows higher redox potential (-0.095 V *vs.* SHE) than the other hemes, although the four hemes in cytochrome c_3 are bis-histidyl hemes (-0.350 V ~ -0.200 V *vs.* SHE). The high redox potential of cytochrome c_3 was similar to that observed for the loss of an axial ligand at heme. To investigate the loss of the histidine ligand, the electrochemistry of four cytochrome c_3 Met mutants, in which the sixth coordinated histidine was replaced by methionine (H22M, H35M, H25M, and H70M), was explored. The electrochemistry of four cytochrome c_3 Ala mutants, in which the sixth coordinated histidine was replaced by

alanine (H22A, H35A, H25A, and H70A), was also explored. As the result, the electrochemistry of the cytochrome c_3 mutants indicated that Heme III alone undergoes loss of its axial histidine ligand.

In addition, kinetic of an intramolecular electron transfer of cytochrome c_3 was investigated by high speed cyclic voltammetry, showing that conformational change of heme II was rate determining step. It means that heme II controls rate of conformational change of cytochrome c_3.

Finally, redox potentials of each heme was successfully clarified by direct electrochemical measurement. The redox reaction of heme follows a conformational change arising from hydrogen bond rearrangement and the redox potentials of four hemes are close to each other, so that individual redox potentials of four hemes are not observed yet. In a previous study, redox titration detected by NMR and ESR only provide the overall redox potential resulting from a conformational change in cytochrome c_3 during the redox reaction. To identify the transient redox potential of each of the four hemes in cytochrome c_3 as the protein changes conformation requires suppression of these conformational changes. In this study, the redox potentials of the four hemes in cytochrome c_3 were investigated by direct electrochemical measurement at -5 °C, super-cooled temperature. To identify the redox potentials of the four hemes, the electrochemistry of four cytochrome c_3 Ala mutants (H22A, H35A, H25A, and H70A) was carried out. The cyclic voltammograms arising from the four hemes are complicated but in the mutants, the redox potential of the one heme in which one histidine ligand is replaced with alanine is obviously higher. Thus, the redox potential of the bis-histidyl coordinated each heme can be determined.

In this study, mechanisms of intramolecular electron transfer of cytochrome c_3 were investigated and the roles of cytochrome c_3 during biological electron transfer were clarified.