II-D Electron Transfer Regulation in Tetraheme Cytochromes *c*

Tetraheme cytochromes *c* are involved in the anaerobic energy metabolism. Cytochrome c_3 (cyt c_3) is an electron transport protein working in strictly anaerobic sulfate-reducing bacteria. This is a small (M.W. ≈ 14,000) soluble protein and shows very low redox potentials (typically, –240 ~ –357 mV *vs.* NHE). We have developed a new expression system using *Shewanella oneidensis*. Using this expression system, now we can examine the role of each amino acid by gene-engineering. At first, we have examined the role of tyrosine-43. We have also determined the complete *g* tensors of the four hemes. Small tetraheme cytochrome *c* (ST cyt *c*) is found in facultative anaerobes of *Shewanella* species and is the smallest tetraheme cytochrome (M.W. ≈ 12,000). The four hemes in ST cyt *c* is arranged in a chain-like manner in contrast to the cyclic heme architecture in cyt c_3. The major aims of this project is to elucidate the mechanism of the regulation of the electron transfer in tetraheme cytochromes *c* on the basis of tertiary structure and heme architecture. For this purpose, we are characterizing two different cytochromes mentioned above by NMR and electrochemistry. Since porphyrin is one of important elemental materials in nano-science, elucidation of the function of particular heme architectures would also contribute to this field.

II-D-1 Role of the Aromatic Ring of Tyr43 in Tetraheme Cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F

OZAWA, Kiyoshi[3]; YASUKAWA, Fumiko[3]; TAKAYAMA, Yuki[2]; OHMURA, Tomoaki[4]; CUSANOVICH, Michael A.[5]; TOMIMOTO, Yusuke[6]; OGATA, Hideaki[6]; HIGUCHI, Yoshiki[6]; AKUTSU, Hideo[1]

([1]IMS and Osaka Univ.; [2]Osaka Univ.; [3]Yokohama Natl. Univ.; [4]Mitsubishi Heavy Industries; [5]Unv. Arizona; [6]Kyoto Univ.)

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Tyrosine 43 is positioned parallel to the fifth heme axial ligand, His34, of heme 1 in the tetraheme cytochrome c_3. The macroscopic and microscopic formal redox potentials of Y43L and Y43F cytochromes c_3 were determined by differential pulse polarography and ^{1}H-NMR. Although the replacement of tyrosine with leucine increased all the redox potentials, the phenylalanine mutation generally did not change them. This strongly suggests that the aromatic ring at this position contributes to lower the redox potentials of cytochrome c_3. The effect of the leucine and phenylalanine mutations on the interacting potential between heme 1 and heme 2 shows that the aromatic ring is also involved in the cooperative reduction of these hemes. Furthermore, temperature dependent line-width broadening in partially reduced samples established that the aromatic ring at position 43 participates in the control of the kinetics of intramolecular electron exchange. The rate of reduction of Y43L cytochrome c_3 by 5-deazariboflavin semiquinone under partially reduced conditions was significantly different from that of the wild-type in the last stage of the reduction, supporting the involvement of Tyr43 in regulation of reduction kinetics. No significant change was found in the crystal structures of the wild-type and Y43L cytochrome c_3.

II-D-2 Correlation between the *g* Tensors and Nonplanarity of Porphyrin Rings in *Desulfovibrio vulgaris* Miyazaki F Cytochrome c_3 Studied by Single Crystal EPR

SAITOH, Takashi[1]; TACHIBANA, Yoshihiko[2]; HIGUCHI, Yoshiki[3]; HORI, Hiroshi[1]; AKUTSU, Hideo[4]

([1]Osaka Univ.; [2]Yokohama Natl. Univ.; [3]Himeji Inst. Tech.; [4]IMS and Osaka Univ.)

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Single crystals of cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F are examined by EPR at cryogenic temperature. The principal values and the eigenvectors are determined. The four sets of EPR signals are directly assigned to the specific four hemes in the three-dimensional structure. The relative energy levels of the three *d* orbitals (d_{xy}, d_{xz} and d_{yz}) of each heme iron calculated from the obtained principal *g* values have shown that the energy gap between d_{xy} and d_π is small for a heme with the S_4-ruffled distortion (heme 1 and heme 2) while the energy gap is large for a heme with the S_4-saddled distortion (heme 4). The determined *g* tensor orientations indicated that the principal *g* axes of heme 1, heme 2, heme 3 and heme 4 co-rotate with the imidazole planes of the sixth ligands.

II-D-3 A Directional Electron Transfer Regulator Based on Heme-Chain Architecture in The Small Tetraheme Cytochrome *c* from *Shewanella oneidensis*

HARADA, Erisa[2]; KUMAGAI, Jiro[3]; OZAWA, Kiyoshi[3]; IMABAYASHI, Shinichiro[3]; TSAPIN, Alexandre S.[4]; NEALSON, Kenneth H.[4]; MEYER, Terrance E.[5]; CUSANOVICH, Michael A.[5]; AKUTSU, Hideo[1]

([1]IMS and Osaka Univ.; [2]Osaka Univ.; [3]Yokohama Natl. Univ.; [4]Cal. Inst. Tech.; [5]Unv. Arizona)

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The macroscopic and microscopic redox potentials of the four hemes of the small tetraheme cytochrome *c* from *Shewanella oneidensis* were determined. The microscopic redox potentials show that the order of reduction is from hemes in the C-terminal domain (hemes 3 and 4) to the N-terminal domain (heme 1),

demonstrating the polarization of the tetraheme chain during reduction. This makes heme 4 the most efficient electron delivery site. Furthermore, multistep reduction of other redox centers through either heme 4 or heme 3 is shown to be possible. This has provided new insights into the two-electron reduction of the flavin in the homologous flavocytochrome *c*-fumarate reductase.