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). 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 Redox-Coupled Conformational Alternations in Cytochrome c_3 from *D. vulgaris* Miyazaki F on the Basis of its Reduced Solution Structure

HARADA, Erisa[1,2]; FUKUOKA, Yuki[2]; OHMURA, Tomoaki[3]; FUKUNISHI, Arima[2]; KAWAI, Gota[4]; FUJIWARA, Toshimichi[1]; AKUTSU, Hideo[1]
([1]Osaka Univ.; [2]Yokohama Natl. Univ.; [3]Mitsubishi Heavy Ind.; [4]Chiba Inst. Tech.)

[*J. Mol. Biol.* **319**, 767 (2002)]

Heteronuclear NMR spectroscopy was performed to determine the solution structure of ^{15}N-labeled ferrocytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF). Although the folding of the reduced cytochrome c_3 in solution was similar to that of the oxidized one in the crystal structure, the region involving hemes 1 and 2 was different. The redox-coupled conformational change is consistent with the reported solution structure of *Desulfovibrio vulgaris* Hildenborough ferrocytochrome c_3, but is different from those of other cytochromes c_3. The former is homologous with *Dv*MF cytochrome c_3 in amino acid sequence. Small displacements of hemes 1 and 2 relative to hemes 3 and 4 were observed. This observation is consistent with the unusual behavior of the 2^1CH_3 signal of heme 3 reported previously. As shown by the ^{15}N relaxation parameters of the backbone, a region between hemes 1 and 2 has more flexibility than the other regions. The results of this work strongly suggest that the cooperative reduction of hemes 1 and 2 is based on the conformational changes of the C-13 propionate of heme 1 and the aromatic ring of Tyr43, and the interaction between His34 and His35 through covalent and coordination bonds. Furthermore, it turned out that the unusual conformational distortion is involved in the attachment of heme 2. This will be associated with the unique structural properties of heme 2 in cytochromes c_3.

II-D-2 A 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]; KUMAGAI, Jiro[3]; 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 Ind.; [5]Unv. Arizona; [6]Kyoto Univ.)

A novel *c*-type multiheme cytochrome overproduction system has been used to prepare large quantities of *Desulfovibrio vulgaris* Miyazaki F cytochrome c_3 and two mutations of the highly conserved aromatic residue, Tyr43. 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 decreased them. This strongly suggests that the aromatic ring at this position is important for maintenance of the extremely low 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 linewidth 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.

II-D-3 A Directional Redox-Regulator Based on the 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)

[*FEBS Lett.* in press]

The macroscopic and microscopic redox potentials of the four hemes of the small tetraheme cytochrome *c* (STC) from *Shewanella oneidensis* were analyzed. The macroscopic potentials range from –248 to –138 mV, which are higher than those of *D. vulgaris* cytochrome c_3. 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), showing the polarization of the tetraheme chain during the reduction. This makes heme 4 the most efficient electron delivery site. The redox characteristics of this heme architecture fit to multistep reduction of other redox centers through either heme 3 or heme 4. This mechanism could successfully elucidate the reduction mechanism of the flavin in fumarate reductase (flavocytochrome *c*). The characteristics of STC are completely different from those of a cyclic heme arrangement in cytochrome c_3. For the first time, the important role of the heme arrangement in a multiheme protein was brought to light.