Regulation Mechanism of Electron Transfer from Cytochrome *c* to Cytochrome *c* Oxidase

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Electron transfer (ET) plays a crucial role in the process of oxidative phosphorylation in the respiratory chain. The electron flow, which leads to transport of protons across the inner mitochondrial membrane and is coupled to synthesis of ATP, is terminated at cytochrome *c* oxidase (C*c*O), where a dioxygen molecule is reduced to two water molecules in a four-electron reduction reaction. The four electrons are consecutively donated by cytochrome *c* (Cyt *c*) a small one-electron carrier. In spite of the biological significance of the ET reaction from Cyt *c* to C*c*O, the detailed regulation mechanism has not yet been fully understood due to the lack of the structural information of the ET complex between Cyt *c* and C*c*O.

One of the keys for elucidating the ET mechanism is to identify the interaction site of the protein-protein complex in the ET reaction. To gain structural insights into the molecular mechanism of this essential ET reaction, we utilized ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled Cyt *c* in the absence and presence of unlabeled C*c*O, and successfully identified the interaction site for C*c*O on Cyt *c* [1].

In the binding of reduced Cyt *c*, where the heme iron is reduced (Fe^{2+}), the chemical shift perturbation clearly showed that the hydrophobic heme periphery and adjacent hydrophobic amino acid residues of Cyt *c* dominantly contribute to the complex formation, whereas charged residues near the hydrophobic core refine the orientation of Cyt *c* to provide well-controlled ET. The hydrophobic region, including one of cysteine residues forming the thioether bonds (Cys17) and isoleucine residues (Ile9, Ile11, and Ile81; yellow in Fig. 1 *a*), would interact with aromatic amino acid residues around the Cu_A site, the supposed electron entry site of the subunit II in C*c*O, to form the direct contact between the two redox centers.

The interaction site is also constituted by positively charged lysine residues (Lys5, Lys7, Lys8, Lys13, Lys86, Lys87, and Lys88) on the exposed heme

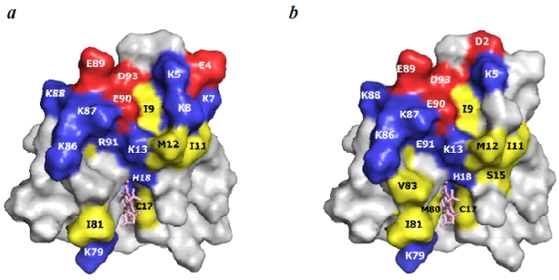

Fig. 1 Interaction Site for C*c*O on Reduced Cyt *c* (*a*) and Oxidized Cyt *c* (*b*)

periphery side (blue in Fig. 1 *a*). In addition to these positively charged residues, negatively charged residues such as glutamic acid (Glu4, Glu89, and Glu90; red in Fig. 1 *a*) were also involved in the interaction site, suggesting that these charged residues regulate the orientation of Cyt *c* on C*c*O to facilitate the ET reaction.

Although such chemical shift perturbations on the ^{1}H-^{15}N HSQC spectrum of ^{15}N-labeled Cyt *c* clearly indicate the interaction site for C*c*O in Cyt *c*, we cannot exclude the possibility that the chemical shift

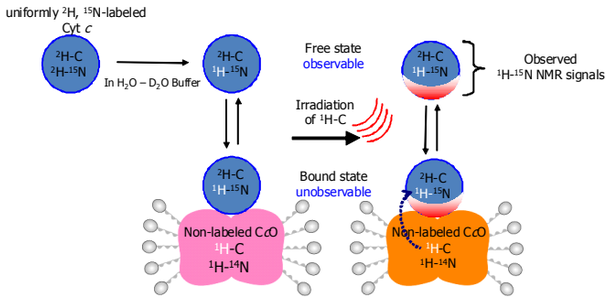

Fig. 2 Transferred Cross-Saturation Measurement

perturbations of the peaks in the ^{1}H -^{15}N HSQC spectrum of Cyt *c* in the presence of C*c*O would not due to the direct interactions with C*c*O, but reflect the secondary effects of the binding of C*c*O on Cyt *c*.

To determine amino acid residues directly interacting with C*c*O, the transferred cross-saturation (TCS) was examined for the complex between Cyt *c* and C*c*O (Fig. 2). Our preliminary results from the TCS measurements suggest that the limited region around exposed heme periphery would be the direct interaction site for C*c*O and some of the charged residues far from the exposed heme periphery such Lys7 and Lys8, which were identified as the amino acid residues interacting with C*c*O by the chemical shift perturbations, would not directly interact with C*c*O.

While the lysine residues far from the exposed heme periphery region would not be involved in the direct interaction site for C*c*O, the Michaelis-Menten parameters, k_{cat} and K_M, for the ET reaction from Cyt *c* to C*c*O clearly showed that the mutational effects at these lysine residues (Lys7, and Lys8) were less drastic, compared with those of the lysine residue near the exposed heme periphery region (Lys13, Lys86, and Lys87), but the mutations significantly reduced the affinity of Cyt *c* to C*c*O. Such significant effects of the lysine residues far from the exposed heme periphery region on the ET activity of Cyt *c* suggest that these lysine residues transiently interact with C*c*O to induce the chemical shift perturbations of the peaks on the ^{1}H-^{15}N HSQC spectrum, but these transient interactions would not be enough to affect the signal intensities on the TCS measurements.

To examine the functional significance of the transiently formed complex between Cyt *c* and C*c*O and further elucidate the molecular mechanism of the ET reaction from Cyt *c* to C*c*O, structural characterization of Cyt *c* and relaxation measurements [2] in the ET complex is now in progress.

Reference

[1] Sakamoto, K, Kamiya, M., Imai, M., Shinzawa-Itoh, K., Uchida, T., Kawano, K., Yoshikawa, S., Ishimori, K. NMR basis for interprotein electron transfer gating between cytochrome *c* and cytochrome *c* oxidase. *Proc Natl. Acad. Sci. USA*, *108*, 12271-12276, (2011).

[2] Sakamoto, K., Kamiya, M., Uchida, T., Kawano, K., Ishimori, K. Redox-controlled Backbone Dynamics of Human Cytochrome *c* Revealed by ^{15}N NMR Relaxation Measurements. *Biophys. Biochem. Res. Commun.* 398, 231-236, (2010)