Low-spin Heme of Cytochrome *c* Oxidase as Driving Element of Proton

Cytochrome *c* oxidase is the terminal enzyme of the respiratory chain, which reduces O_2 to water molecules. The O_2 reduction site of mitochondrial cytochrome *c* oxidase is composed of a high-spin heme (heme a3) and a copper ion (CuB). The protons used for water formation from O_2 are transferred from the inside of the mitochondrial inner membrane (the matrix space) through two hydrogen bond networks known as the K- and D-pathways (Fig. 1). In addition to the transfer of protons to the O_2 reduction site, proton pumping from the matrix space to the intermembrane space through the enzyme results in a proton motive force required to drive ATP synthase.

The X-ray structures of bovine heart cytochrome *c* oxidase in the fully oxidized and reduced states at 2.3 and 2.35 Å resolution, respectively, indicate the movement of Asp51 of subunit I (the largest subunit containing heme *a* and the O_2 reduction site [1]), from the interior of the protein to the intermembrane surface upon the reduction of the enzyme [2]. In the oxidized state, Asp51 makes contact with the matrix space via a hydrogen bond network with a channel in which water molecules in the matrix space are accessible (H-pathway) (Fig. 2). These structures are highly indicative of a proton-pumping event occurring at Asp51 [2].

Fig. 1. Protons are transferred to the O_2 reduction site through two hydrogen bond networks known as the K- and D-pathways.

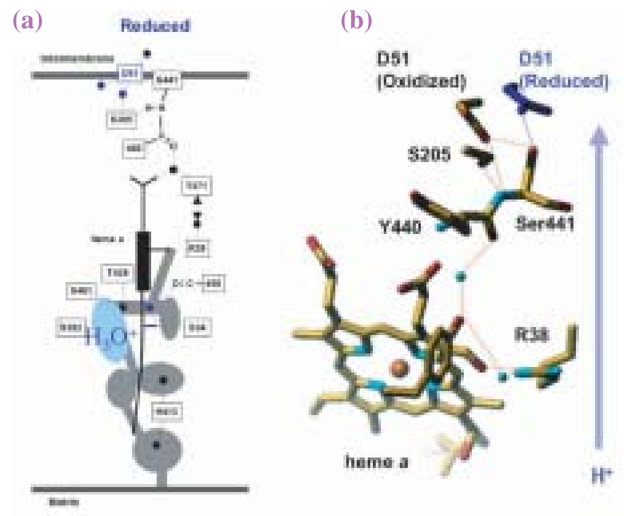

Fig. 2. **(a)** Schematic drawing of H-pathway through which protons are pumped, and **(b)** structure of upper half of H-pathway.

However, the proton-pumping proposal is not yet widely accepted because plant and bacterial enzymes do not have an analogous Asp51 (bovine numbering) residue and because a D-pathway mutation results in a bacterial enzyme as described below. Mutations of amino acid residues within the D-pathway cause a decrease in proton pumping efficiency and O_2 reduction activity [3], observations apparently consistent with a process wherein proton transfer for proton pumping occurs via the proton pathway for water molecule formation. Furthermore, no mechanisms for driving the proton pump at Asp51 have yet been determined. The mutation of Asp51 would provide one of the most direct methods of probing the function of Asp51. However, the site-directed mutagenesis of subunit I, a large transmembrane subunit encoded by a mitochondrial DNA, has not been attempted due to expected technical difficulties [4].

We successfully investigated the role of the H-pathway by X-ray crystallography (**BL44XU**), infrared spectroscopy and site-directed mutagenesis [5]. The X-ray structures of bovine cytochrome *c* oxidase in the oxidized and reduced states at 1.8 and 1.9 Å resolutions, respectively, indicate redox-coupled changes in the conformation of the heme *a* hydroxyfarnesylethyl group (Fig. 3), resulting in an alteration of the capacity of a water channel that connects the matrix surface with the heme a formyl group. A redox-coupled change in the capacity of the water channel of the H-pathway, induced by the hydroxyfarnesylethyl group of the low-spin heme, suggests that the channel functions as an effective proton-collecting region.

The hydrophobicity of the Asp51 environment near the intermembrane surface is also altered. Infrared spectroscopy results indicate that the conformation of Asp51 is controlled only by the oxidation state of the low-spin heme. A hydrogen bond network connects Arg38 with Asp51. Furthermore, an Asp51Asn mutation abolishes the proton-pumping function without impairing O_2 reduction activity. These results indicate that the low-spin heme drives the proton-pumping process via the H-pathway.

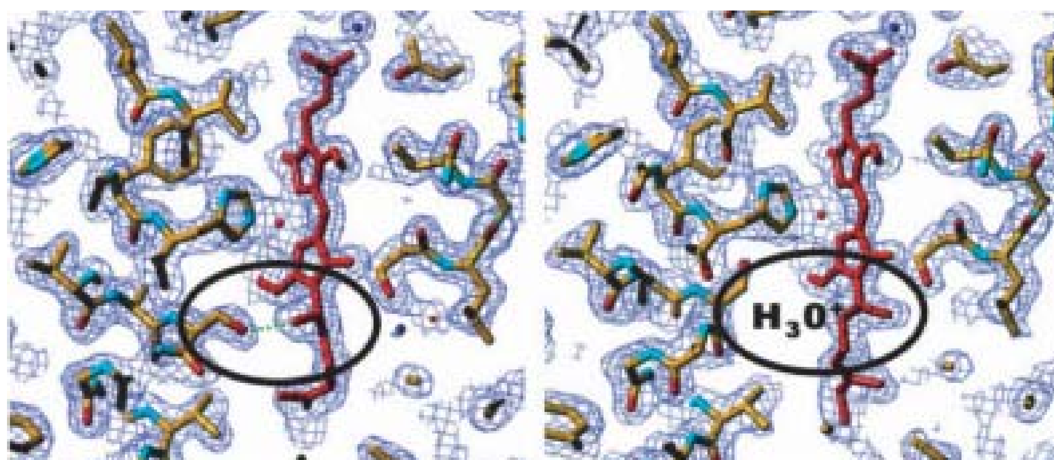

Fig. 3. Structural change of heme *a* hydroxyfarnesylethyl group upon reduction of heme.

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References

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