Redox-coupled Structural Changes in Bovine Heart Cytochrome c Oxidase

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Cytochrome c oxidase, a key enzyme in cell respiration, catalyzes the reduction of dioxygen to water using protons extracted from the matrix side of the inner mitochondrial membrane and electrons from cytochrome c, in a reaction that is coupled with proton pumping. The crystal structures of an eukaryotic and prokaryotic cytochrome c oxidase reported at 2.8-Å resolution in 1995 ushered in a new era for cytochrome c oxidase research.[1,2] In 1996, the protein structure, phospholipids, and possible nucleotide binding sites of the bovine heart enzyme were reported in detail. In addition, the eukaryotic protein structure revealed two possible proton-pumping paths neither of which involves the dioxygen reduction site.[3] In contrast, a scheme of the redox-coupled proton pumping, in which an imidazole ligand of CuB plays a crucial role, has been proposed based on the crystal structure of the bacterial enzyme.[2] For the proton-pumping function of cytochrome c oxidase driven by the dioxygen reduction, an acidic group in the protein must be accessible to only one of the two bulk water phases on both sides of the mitochondrial membrane in a certain oxidation state of the enzyme, and the accessible side must be switched to the other side by change in the oxidation state, concomitantly with a significant change in pK of the acidic group. Here we report crystal structures of the fully oxidized form at 2.30-Å resolution, the fully reduced form at 2.35-Å resolution, and the azide and CO complexes at 2.9- and 2.8-Å resolution, showing significant redox-coupled conformational changes in the segment containing Asp51 of subunit I and at CuB site. The crystal structures of fully oxidized and fully reduced bovine heart cytochrome c oxidase reveal new aspects for the enzymatic mechanism. A covalent link between Tyr244 and His240 suggests an increase in the acidity of Tyr244-OH group. Thus, Tyr244 should be the proton donor to the bound dioxygen to form a hydroperoxo intermediate (Fe-OOH). In the fully oxidized state, Asp51 is completely buried inside the protein and is connected with the matrix surface by a hydrogen bond network. The residue migrates to the cytosolic surface upon reduction, disrupting the hydrogen bond network. The movement indicates a novel proton-pumping mechanism.

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[1]T.Tsukihara, et al., Science, 269, 1069(1995).
[2]S.Iwata, C.Ostermeier, B.Ludwig, H.Michel, Nature, 376, 660(1995).
[3]T.Tsukihara, et al., Science, 272, 1136(1996).