Poster Presentation

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Structures of bovine cytochrome oxidase reveal proton active transports

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Bovine cytochrome c oxidase (CcO) pumps four protons in each catalytic cycle through H-pathway including a hydrogen-bond network and a water channel in tandem. Protons, transferred through the water-channel from the negative side of mitochondrial inner membrane into the hydrogen-bond network, are pumped to the positive side of the membrane electrostatically by net positive charges on a heme (heme a) iron created upon electron transfer to the O2 reduction site. For blockage of backward proton leak from the hydrogen-bond network, which determines the proton-pumping direction, the water channel is closed after O2 binding to initiate proton-pump. Thus, four protons must be collected in the hydrogen-bond network before O2 binding. The X-ray structural analyses of the oxidized/reduced CcO at 1.5/1.6 Å resolution reveal a large cluster composed of ~21 water molecules and a Mg2+ site including Glu198 (Subunit II) bridging CuA and Mg2+. The cluster of the oxidized state have 20 water sites with full occupancy and two sites with partial occupies of water, while that of the reduced state have 19 water sites with full occupancy and 3 sites with partial occupancies. The carboxyl group of Glu198 changes its coordination structure to Mg2+ upon the reduction of the active centers. The cluster is tightly sealed sterically against proton exchanges with the cluster outside except for a short hydrogen-bond network connecting the cluster with H-pathway. Five proton-acceptable groups hydrogen-bonded with the cluster suggest sufficient storage capacity for four proton equivalents. The redox-coupled structural changes in the electron transfer pathway from CuA, the initial electron acceptor from cytochrome c, to heme a suggest redox-driven effective proton donations from the cluster to H-pathway, facilitated by Glu198. These results indicate that the cluster is a crucial element of the proton-pumping system of bovine CcO.

Keywords: membrane protein, proton transfer, enzymatic mechanism