method is generic and can be applied to any system composed of rod-shaped molecules.

The X-ray scattering experiments were performed at ESRF microfocus beamline ID13 on collagen tendon fibers extracted from mouse tails.

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Keywords: collagen, paracrystal, modeling

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Structural changes of bovine cytochrome *c* oxidase dependent on the redox states

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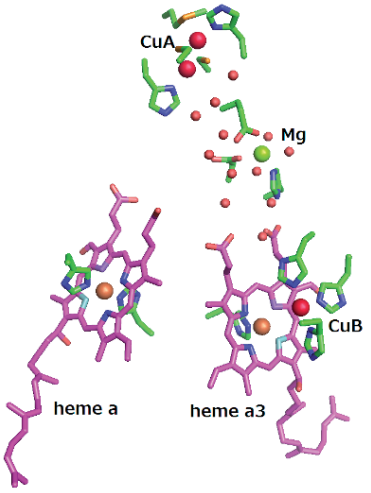
Cytochrome *c* oxidase (CcO) is a terminal oxidase in the respiratory chain. Mitochondrial CcO contains four redox active metal sites, CuA, heme a, heme a3 and CuB. The oxygen molecule is bound to the reduced heme a3-CuB site and reduced to water molecules by electrons from cytochrome c. The oxygen reduction is coupled to the proton translocation across the membrane, generating electrochemical proton gradient. To understand energy transducing mechanism, we have been determined the structural changes of bovine mitochondrial CcO in the resting oxidized and the reduced states. In previous analyses, the conformational changes were found at the peptide segment containing aspartate residue on the CcO surface and the α-helix between hemes a and a3 [1, 2]. It has been suggested that these changes contribute to the proton transfer.

In this study, we prepared bovine CcO crystals in the reduced state which was identified by the measurement of the UV/vis absorption spectrum of the single crystal. We performed X-ray diffraction experiment at BL44XU in SPring-8 and determined the detailed structural changes at 1.4 Å resolution. The FO-FO electron density map calculated by using the data from the resting oxidized and the reduced states indicates that the changes are localized at the inside of CcO, where the redox active metal sites are located. In addition to the previously found conformational changes, it is found that water molecules in the water cluster between subunit I and II are moved dependent on the redox states. These waters are located near the CuA site in the subunit II and the Mg site which is octahedrally coordinated by one aspartate residues in the subunit II, one aspartate and one histidine residues in the subunit I and three water molecules. Furthermore, refined structural changes were found at heme a3 porphyrin plane and the CuB ligand. It is suggests that these changes are involved in the energy transducing reaction.



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High resolution analysis and anomalous dispersion analysis of bovine cytochrome *c* oxidase

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Cytochrome *c* oxidase (CcO) in the respiratory chain is supramolecular membrane protein. CcO functions as a terminal oxidase catalyzing the molecular oxygen reduction coupled to the proton transfer across the membrane. The X-ray structures of mitochondrial and several bacterial CcOs were determined and its resolutions have been improved step by step [1-2].

To understand proton transfer mechanism, we aim to determine orientations and protonation states of amino acid residues (imidazole and carboxyl group etc.) in the proton pathways. In this study, we performed X-ray diffraction experiment at BL44XU in SPring-8 using bovine mitochondrial CcO crystals in the resting oxidized state and improved the resolution to 1.4 Å. To reduce X-ray irradiation damage, diffraction data were collected from many isomorphous crystals. To improve accuracy of the data, the redundancy of data was increased and low quality images (R_{merge} > 0.3 or mosaicity > 0.3) were removed. Resultant data shows 1.6 of I/ σ (I) at 1.42-1.40 Å resolution range. Electron density map shows many multi-conformations of amino acid residues (Met etc.). Structures of phospholipids and ligand at the O_2 reduction site are refined.

CcO crystals give anisotropic diffraction intensities dependent on the crystal axis and the direction of X-ray beam. Processing of the diffraction images at anisotropic resolution ranges improved R and R_{free} values without lowering quality of the electron density.

Anomalous scattering analysis was carried out to determine phosphorus atom positions in phospholipids and to identify the atomic species of the ligand at the O_2 reduction site. The difference anomalous peaks indicate that 7 phospholipids are located on the CcO surface, so we correct previous models [3]. No difference anomalous peak was observed at the O_2 binding site indicating that the ligand is not chloride ion.