Mechanism of Proton Pumping of Cytochrome c Oxidase based on its Crystal Structure

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The purpose of this project is to get crystallographic structural information on the reaction intermediates of the dioxygen reduction by cytochrome c oxidase which is indispensable for elucidation of the mechanism of the proton pumping driven by the dioxygen reduction. We have obtained the crystal structures of fully oxidized, fully reduced, CO bound fully reduced, azide bound fully oxidized and cyanide bound fully oxidized forms at 2.3Å, 2.35Å, 2.8Å, 2.9Å and 2.8Å resolution, respectively. From these structures, we have identified a redox coupled conformational changes of an aspartate residue which are likely to induce a redox coupled changes in the effective accessibility to the bulk water phases and in pK. The conformational change indicate that the aspartate is the site for the proton pumping. In order to elucidate the mechanism of coupling of the dioxygen reduction and the proton pumping reaction, we have to know crystal structure of the intermediate species during the dioxygen reduction.

We have established the method for preparing the fully reduced enzyme crystals from the fully oxidized crystals by treating with ascorbate-cytochrome c system at room temperature without damaging the quality of the crystals. However, in order to prepare the crystals of the intermediate species during the dioxygen reduction, the intermediate species must be stabilized by lowering the temperature. Thus, we tried to establish the conditions for freezing the fully reduced crystals placed in an X-ray diffraction capillary filled with mother liqueur containing a reducing system. We have found conditions for freezing crystals placed in an X-ray diffraction capillary with liquid nitrogen with essentially the same method as for the crystals placed in the loops for flash cooling. The quality of the crystals was not significantly impaired by the freezing process. That is, the frozen crystals of the fully reduced form diffracted X-rays up to 2.5 Å resolution, though the highest resolution were dependent on the angles of the X-ray diffraction. The biggest problem we have found in the X-ray diffraction experiments performed in this project is that the crystals once frozen are no longer isomorphous to the crystals before freezing. Furthermore, the effect of freezing is not reproducible, that is, frozen crystals are not isomorphous with each other. We are improving the frozen conditions.