High Resolution X-ray Crystallographic Analysis of Cytochrome c Oxidase

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Crystal structure of cytochrome c oxidase at high resolution is indispensable for elucidation of the mechanism of the proton pumping driven by the dioxygen reduction. The redox coupled conformational change which induces the proton pumping could be very small. Thus, it is impossible to estimate the resolution of the crystal structure high enough for identification of the redox coupled conformational changes at the proton pumping site.

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. However, we have to know the structural change in more detail for elucidation of the function of the aspartate residues in the proton pumping. Furthermore, no structural change which couples the dioxygen reduction at heme a3 with the proton pumping at the aspartate residue has not been detected, because the resolution of the crystal structure is not high enough. Thus, the purpose of this project is to improve the resolution of the crystal structure of the fully oxidized bovine heart cytochrome c oxidase by collecting X-ray diffraction data from the crystals frozen at liquid nitrogen temperature.

We tried to establish the conditions for freezing the fully oxidized crystals with a flash cooling under liquid nitrogen temperature. After an extensive search for the conditions for freezing crystals in our laboratory, we have found conditions for freezing crystals placed in 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 oxidized form diffracted X-rays up to 2.0 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 now improving the frozen conditions. However, the crystals which we used for the diffraction experiment in Spring-8 were those which diffract X-rays up to about 3 Å resolution under unfrozen conditions using PF. Thus, the present result indicates that X-ray diffraction experiments in Spring-8, under the frozen conditions improves the resolution dramatically.