X-ray Crystallographic Study of the Closed Form of Citrate Synthase from *Thermus thermophilus* HB8

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Citrate synthase (E.C. 4.1.3.7), an enzyme involved in the first step of tricarboxylic acid cycle, catalyzes the condensation of citrate from acetyl-coenzyme A and oxaloacetate. Previous crystallographic studies of citrate synthase from pig have shown that large scale conformational changes are induced upon binding of the ligands. To better understand the molecular mechanism of this enzymatic reaction, we have performed a structural study of citrate synthase (*Tt*CS) from thermophilic eubacteria, *Thermus thermophilus* HB8.

*Tt*CS functions as a homodimer of Mr 85,000, with each monomer comprising 377 amino acids. We previously obtained a tetragonal crystal that belongs to the space group $P4_32_12$ (a = b = 84.55 Å, c=153.52 Å) and determined the structure of the ligand-free protein at 1.5 Å resolution (2000B0243-NL –np). Here we report our recent structural investigation on the closed form of *Tt*CS.

*Tt*CS used in this study was prepared by Kuramitsu's group (RIKEN Harima Institute). When the purified enzyme was incubated with citrate and CoA and then crystallized using lithium sulfate as precipitant, an orthogonal crystal grew in a few days. This crystal belongs to the space group $P2_12_12_1$ with unit cell dimensions of a = 83.2 Å, b = 112.3 Å and c = 187.2 Å. Diffraction data at 2.3 Å resolution suggested that two dimers exist in the asymmetric unit. The self-rotation function (Fig.1) indicates that the non-crystallographic 2-fold symmetry axis forms an angle of 30° or 60° with the c axis. The molecular replacement analysis using the open form of *Tt*CS as a search model indicated that the NCS axis lies between the neighboring dimers in the asymmetric unit.

When PEG4000 was used as precipitant, *Tt*CS crystallized with citrate and CoA into a monoclinic form. The diffraction data at 3 Å resolution indicated that this crystal belongs to the space group C2 with cell dimensions of a = 149.3 Å, b = 241.1 Å, and c = 80.9 Å ($\alpha = \gamma = 90°$, $\beta = 111°$). The Matthews number V_M is calculated to be 4.02 - 1.61 if the asymmetric unit contains 2 - 4 dimers.

We also obtained a triclinic crystal (a = 60.6 Å, b = 103.6 Å, c = 116 Å, $\alpha = 68°$, $\beta = \gamma = 76°$). This crystal grew in the presence of oxaloacetate and CoA, and diffracted X-rays up to 2.3 Å resolution. Assumption of 2 – 4 dimers in the asymmetric unit gives V_M of 3.8 – 1.9.

To determine the structure of the closed form of *Tt*CS, we are now carrying out a crystallographic analysis of the $P2_12_12_1$ crystal. Our current model of the closed form suggests that binding of the ligands (citrate and CoA) causes a large rotation of the small domain with reference to the large domain.

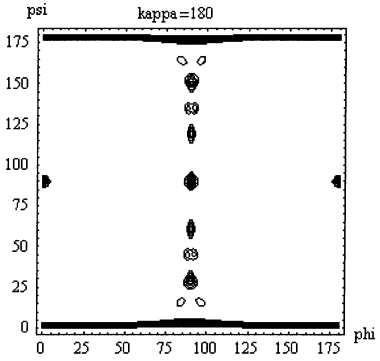

Fig1. Self-rotation function calculated for diffraction data of the $P2_12_12_1$ crystal.

X-ray Crystallographic Study of Light-Harvesting Chlorophyll a/b Protein Complex

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In photosynthetic systems of higher plants, an integral membrane protein called Light Harvesting Chlorophyll a/b protein Complex (LHC-II) is responsible for the absorption of light energy. When antenna pigments (chlorophyll a, b and carotenoids) in LHC-II absorb light, the absorbed energy is rapidly transferred to adjacent pigments and eventually to the reaction center. The major role of the polypeptide chain is to keep the antenna pigments in their proper location and orientation. Construction of its atomic-resolution structural model will extend our understanding of the energy transfer mechanism in the photosynthetic system.

We previously developed a novel crystallization method to obtain a high-quality crystal of the membrane protein bacteriorhodopsin, and determined its structure at 2.5Å resolution. Our interest is to examine whether our crystallization method can be applied to other membrane proteins. By applying this method, we have recently obtained octahedral crystals and hexagonal crystals of LHC-II. The former crystal was shown to be made up of the icosahedral assembly of LHC-II. The hexagonal crystal, which is suggested to be composed of stacked membranes, appears to be more suitable for high-resolution structural analysis. Using this crystal form, we have challenged to determine the structure of LHC-II at high resolution. Our previous study indicated that the hexagonal crystal diffracted up to 2.2Å resolution. However, the observed diffraction spots were diffuse and diffraction pattern exhibited anisotropic feature. To improve the quality of the crystal, we have carried out the refinement of the crystallization condition. In this study, we used the hexagonal crystal grown by using PEG 1,000 as precipitant. The crystal size was typically 500×500×5 μm^3. Crystals were flash-frozen by liquid propane.

When the center of the crystal was irradiated by the X-ray beam, the observed diffraction spots were streaked badly in the direction of the c*-axis. Sharp diffraction spots were observed when the x-ray beam was passed through the edge of the crystal. So we collected diffraction data using the edge of the crystal. A typical example of diffraction image is shown in Fig.1. The crystal diffracted anisotropically to 4Å resolution in the best direction. Collected data were indexed and integrated by DPS-MOSFLM ver2.0. The crystal was shown to belong to space group $P6_322$ with the unit cell dimensions of a=b=128.55Å, c=134.19Å. Because of the highly anisotropic mosaicity, the R_{merge} of the scaled data was high (0.177, completeness: 77.2%). To improve the data statistics, we need to modify the integration software.

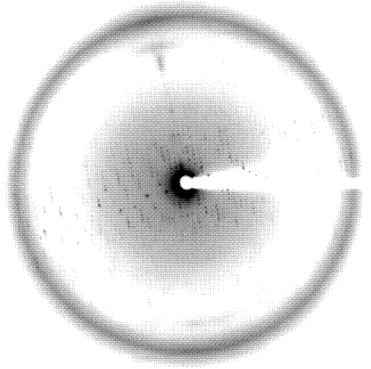

Figure 1. Diffraction image of the hexagonal crystal of LHC-II