Crystallographic Analysis of Proteins Related to Drug Design IX

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The fatty acid β-oxidation multienzyme complex from *Pseudomonas fragi*, HDT, exhibits predominantly the three enzyme activities of 2-enoyl-CoA **h**ydratase, 3-hydroxyacyl-CoA **d**ehydrogenase and 3-oxoacyl-CoA **t**hiolase. The goal of this study is to elucidate the sequential catalytic reaction mechanisms of the multienzyme complex at the atomic level. Taking its relatively small molecular mass and high stability into account, the HDT complex should be the most appropriate target for a structural study of the multienzyme complex, using the combined techniques of protein engineering and X-ray crystallography. Using this beamtime, we collected the diffraction data of two native and a heavy atom (Au) derivative crystals.

The recombinant *P. fragi* HDT was over-expressed using a *E.coli* expression system, and was purified by chromatography to homogeneity. The native crystals were obtained by the hanging-drop vapor diffusion method using polyethyleneglycol 4000 as a precipitant. The gold derivative crystals were prepared by the co-crystallization with 0.06 mM $NaAuCl_4$. The crystals belong to the space group $P2_12_12_1$ with a cell dimension of a= 96 Å, b= 136 Å, c= 197 Å at 100 K. They diffract X-ray beyond 3 Å resolution. However, the unit cell dimensions drastically change (5-30 Å) from crystal to crystal under the condition of cryoprotection. The data collection was performed at BL24XU using the R-AXIS IV detector. A typical size of the crystals used for data collection was 50 μm × 150 μm × 300 μm. The raw data were digitized and marged using the programs *DENZO* and *SCALEPACK*. The R-merge of the native data was 7.7 %. Unfortunately, the phasing by the isomorphous replacement method was unsuccessful because of the non-isomorphism of the data. We are now searching the cryoprotection condition to stabilize the crystals.