X-Ray Crystallographic Study of acyl-CoA Oxidase

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Mammalian acyl-CoA oxidase (ACO) catalyzes the first and rate-limiting step of the peroxisomal β-oxidation of fatty acids, which is one of the two β-oxidation systems of mammalian origin, the other being mitochondrial acyl-CoA dehydrogenase (ACD). We have recently cloned the cDNA for rat liver ACO and expressed in *Escherichia coli*. Rat liver ACO is a flavoenzyme comprising 661 amino acid residues and a flavin-adenine dinucleotide (FAD) per subunit. The sequence alignment between ACDs and ACOs reveals that they are derived from a common ancestral protein and that they belong to the same superfamily. The three-dimensional structure of ACO will provide critical information in understanding the reaction mode of ACO toward both acyl-CoA and molecular oxygen. It will further subsidize the knowledge of the reaction of the ACD/ACO superfamily as a whole.

The yellow crystals of ACO appeared within a week of incubation and grew to a maximum size of 0.20 × 0.15 × 0.40 mm. From the diffraction data, the space group of the crystal was determined to be orthorhombic $P2_12_12_1$. Assuming one dimer in the asymmetric unit, the Matthews coefficient V_m value was calculated as 2.21 Å^3 Da^{-1} indicating a solvent content of approximately 44 % in the unit cell

For data collection, yellow crystals were soaked for a few seconds in a solution containing 30 %(w/v) PEG400, 8 %(w/v) PEG20000, 100mM potassium phosphate (pH 7.4). The crystals were then mounted in a 0.5mm cryoloop, and flash-frozen in liquid nitrogen stream at 100 K. The data set for a crystal soaked in 0.1mM chloromethyl-mercury was collected at 2.6 Å (R_{merge} = 7.0 %) resolution. The crystal showed no significant decay upon exposure. Four mercury sites of the chloromethylmercury derivative were located using the difference Patterson maps. We are now in the process of refining the heavy-atom parameters for the derivative for determination of three dimensional structure of rat ACO.

CH_3HgCl derivative

Space group	$P2_12_12_1$
Unit-cell parameter	a=71, b = 91, c=214Å
Crystal size	0.2×0.1×0.3 mm
Resolution range	20.0～2.6 Å
No. of reflections	200374
No. of unique reflections	44225
Completeness (%)	99.9
R_{merge}(%)	6.7

X-ray Crystallographic study of Serine Racemase

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Free D-serine has been found to be confined predominantly to the forebrain structure of mammals and persists at high levels throughout embryonic and postnatal life. Several reports strongly suggest that serine racemase exists in mammalian brain and produces D-serine, an endogenous ligand for *N*–Methyl–D–aspartate receptor. Serine racemase requires pyridoxal 5'-phosphate and catalyzes racemization from L-Serine to D-Serine. Suprisingly, the distribution of putative serine racemase is not limited to higher animals. Mouse serine racemase might fall on a new class of pyridoxal amino acid racemases, which is distinct from both bacterial and fungal alanine racemases, but similar to bacterial threonine dehydratase in primary structure.

S. pombe gene homologous with mouse serine racemase is overexpressed in *E.coli*. The enzyme is purified and crystallized. Crystals were grown to dimensions of about 0.1 x 0.1 x 0.1 mm by vapor diffusion, belonging to the space group *C*2 with one subunits in the asymmetric unit. Diffraction data collected from a single crystal at 100K using 1.0 Å radiation. Rotation data were recorded on MAR CCD detector. Data set was processed and scaled using the MOSFLM program package as is shown in Table 1.

Table 1.

Cell constant	
a (Å)	58.58
b (Å)	73.16
c (Å)	64.81
β (°)	100.88
Resolution (Å)	2.3
Observed reflections	146,891
Unique	12,014
Completeness (%)	98.6
R_{merge} (%)	8.5
I/sigma (I)	6.9

Molecular replacement calculations were carried out using the program AMoRe implemented in the CCP4 suite using threonine dehydratase from *E. coli* as a starting model. One marked solution was obtained for the model and the electron density maps calculated from the model gave the protein core resion. However, the overall protein model was not built, and a partial model gave high free-R value. Now, we search several heavy atom derivatives for MIR method.