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Crystallization and preliminary X-ray characterization of rat liver acyl-CoA oxidase

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A recombinant form of the flavoenzyme acyl-CoA oxidase from rat liver has been crystallized by the hanging-drop vapour-diffusion technique using PEG 20 000 as a precipitating agent. The crystals grew as yellow prisms, with unit-cell parameters $a = 71.05$, $b = 87.29$, $c = 213.05$ Å, $\alpha = \beta = \gamma = 90^\circ$. The crystals exhibit the symmetry of space group $P2_12_12_1$ and are most likely to contain a dimer in the asymmetric unit, with a V_M value of 2.21 Å^3 Da^{-1}. The crystals diffract to a resolution of 2.5 Å at beamline BL6A of the Photon Factory. Two heavy-atom derivatives have been identified.

1. Introduction

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) (Kunau *et al.*, 1995). The presence of two forms of ACO has been implied on the basis of the structures of the ACO gene and cDNA (Miyazawa *et al.*, 1987; Osumi *et al.*, 1987). We have recently cloned the cDNAs for two forms of rat liver ACO (ACO-I and ACO-II) and expressed them separately in *Escherichia coli* (Setoyama *et al.*, 1995). The expressed ACO-I and ACO-II exhibited different substrate specificities: ACO-I shows optimal activity towards substrates with acyl-chain length C_8–C_{12} and ACO-II towards substrates with C_{12}–C_{16} chain length (Setoyama *et al.*, 1995). Rat liver ACO-I or ACO-II is a flavoenzyme comprising 661 amino-acid residues and a non-covalently bound flavin-adenine dinucleotide (FAD) per subunit. ACO catalyzes O_2-dependent dehydrogenation of an acyl-CoA to the corresponding *trans*-enoyl-CoA.

Even though both ACD and ACO catalyze dehydrogenation of acyl-CoA to *trans*-enoyl-CoA at the expense of FAD reduction in the reductive half-reaction, reduced FAD in the former transfers electrons to electron-transferring flavoprotein in the oxidative half-reaction, while reduced FAD in the latter reduces molecular oxygen to hydrogen peroxide (Kunau *et al.*, 1995). Moreover, sequence alignment between ACDs and ACOs reveals that they are derived from a common ancestral protein and that they belong to the same superfamily (Matsubara *et al.*, 1989). The structural and enzymological as well as physicochemical properties of ACDs have been extensively investigated (Engel, 1992), whereas the three-dimensional structure of ACO is not available and enzymological and other physicochemical studies on ACO are relatively sparse. 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 strengthen knowledge of the reaction of the ACD/ACO superfamily as a whole as well as knowledge of the mechanism by which different acyl-CoAs are oxidized by one or other of the mitochondrial and peroxisomal β-oxidation pathways. We report here the crystallization and preliminary X-ray crystallographic results of ACO-II.

2. Experimental

2.1. Protein purification

Rat liver ACO-II was expressed in *E. coli* (Setoyama *et al.*, 1995). The cells were suspended in lysis buffer (50 m*M* potassium phosphate, 0.3 m*M* EDTA, 25 µ*M* FAD pH 7.6) and were homogenized by sonication. The cell lysate was centrifuged at 17 000*g* for 60 min to remove cell debris. The cell extract was heated at 321 K for 5 min and fractionated with 30% saturated ammonium sulfate. The enzyme was then purified by a two-step procedure of column chromatography, first with a DEAE-Toyopearl column (TOSOH) equilibrated with 10 m*M* potassium phosphate buffer pH 7.6 using a linear gradient of 0–50 m*M* KCl and then with a DEAE-Toyopearl column equilibrated with 10 m*M* potassium phosphate buffer pH 8.0 using a linear gradient from 0 to 100 m*M* KCl. The purified enzyme was homogeneous on SDS–PAGE. The purified enzyme was concentrated to about 6 mg ml^{-1} concentration using a Centricon 30 (Amicon) and stored in 50 m*M* potassium phosphate buffer pH 7.4 at 253 K.

Figure 1
Crystal of rat liver acyl-CoA oxidase-II. The crystal was grown in a hanging drop using PEG 20 000 as precipitant.

2.2. Crystallization

ACO-II crystals were grown by the hanging-drop vapour-diffusion method at 293 K. The initial screening for crystallization conditions was performed using the sparse-matrix screens I and II from Hampton Research (Jancarik & Kim, 1991). Several crystal forms were obtained and one of the most promising crystallization conditions was optimized. A 10 µl droplet of protein solution [2.0 mg ml^{-1} protein, 3.0%(*w*/*v*) PEG 20000, 20 m*M* potassium phosphate pH 7.4] was equilibrated against 400 µl of reservoir solution [8–11%(*w*/*v*) PEG 20 000, 100 m*M* potassium phosphate pH 7.4] to give crystals of ACO-II.

2.3. Data collection

For preliminary characterization, crystals were mounted in glass capillaries with a small amount of mother liquor; intensity data for unit-cell parameter and space-group determination were collected on an R-AXIS IIc image-plate detector with graphite-monochromated Cu $K\alpha$ radiation from a Rigaku rotating-anode generator operated at 40 kV and 100 mA. Data collection was performed at 100 K using a wavelength of 1.00 Å from the Synchrotron Radiation Source at the Photon Factory BL6A or BL18B with an ADSC Quantum 4R CCD detector system (High Energy Accelerator Research Organization, Tsukuba, Japan). Before flash-freezing, the yellow crystals were soaked for a few seconds in a solution containing 30%(*v*/*v*) PEG 400, 11%(*w*/*v*) PEG 20 000, 100 m*M* potassium phosphate pH 7.4. The crystals were then mounted in a 0.5 mm cryoloop (Hampton Research) and flash-frozen in a liquid-nitrogen stream at 100 K. The data were processed using *MOSFLM* (Leslie, 1992) and *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

Table 1
Crystal data and intensity statistics.

Values in parentheses are for the last resolution shell.

Data set	Native	CH_3HgCl	PCMBS†
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	*a* = 71.1, *b* = 87.3, *c* = 213.1	*a* = 71.9, *b* = 91.4, *c* = 214.3	*a* = 71.0, *b* = 87.6, *c* = 213.0
Temperature (K)	100	100	100
Wavelength (Å)	1.00	1.02	1.00
Resolution range (Å)	20.0–2.5 (2.64–2.5)	20.0–2.6 (2.69–2.6)	20.0–2.8 (2.95–2.8)
No. of reflections	211138 (25993)	200374 (17452)	125745 (16325)
No. of unique reflections	43259 (5520)	44225 (4384)	32497 (4594)
Completeness (%)	93.3 (82.4)	99.9 (99.9)	97.3 (95.0)
R_{merge}‡ (%)	9.4 (21.5)	6.7 (13.9)	7.0 (15.4)
Mean $I/\sigma(I)$	5.8 (3.1)	18.7 (8.4)	7.4 (4.6)

† *p*-Chloromercuribenzene sulfonate. ‡ $R_{merge} = \sum_{hkl}\sum_i |I_{hkl,i} - \langle I_{hkl}\rangle| / \sum_{hkl}\sum_i I_{hkl,i}$, where I is the observed intensity and $\langle I\rangle$ is the average intensity for multiple measurements.

3. Results and discussion

Yellow crystals of ACO-II appeared within a week of incubation and grew to maximum dimensions of 0.3 × 0.2 × 0.5 mm (Fig. 1). From the diffraction data collected on the R-AXIS IIc, 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 was calculated to be 2.21 Å^3 Da^{-1}, indicating a solvent content of approximately 44% in the unit cell. These values are within the range typical for protein crystals (Matthews, 1968). A native data set with 43 529 unique reflections has been collected, giving a data-set completeness of 93.3% in the resolution range 20.0–2.50 Å with an R_{merge} of 9.4% (Table 1). These data indicate good quality of the crystals for X-ray structural analysis. The crystals showed no significant decay upon exposure.

The data sets for crystals soaked in 0.1 m*M* chloromethylmercury for 4 h and 0.1 m*M* *p*-chloromercuribenzenesulfonate for 2 h were collected at 2.6 Å (with R_{merge} = 6.7%) at the Spring-8 BL41XU with a MAR CCD165 detector system and at 2.8 Å (R_{merge} = 7.0%) resolution at the Photon Factory BL18B using the same system as for native crystals, respectively. Four mercury sites of the chloromethylmercury derivative and two mercury sites of the *p*-chloromercuribenzenesulfonate derivative were located using the difference Patterson maps. We are now in the process of refining the heavy-atom parameters for the two derivatives for determination of the three-dimensional structure of rat liver ACO-II.

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