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Crystallization and preliminary X-ray crystallographic studies on recombinant human carnitine acetyltransferase

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In this paper, the purification, crystallization and preliminary X-ray crystallographic studies of human carnitine acetyltransferase are reported. Recombinant human carnitine acetyltransferase crystals were grown by the hanging-drop vapor-diffusion method and belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 137.65, b = 84.76, c = 57.65 Å and one molecule per asymmetric unit. The intensity data were collected from a cryocooled crystal to 1.6 Å resolution using a conventional X-ray source.

1. Introduction

Carnitine acetyltransferase (EC 2.3.1.7; CAT) catalyzes the reversible transfer of short-chain (C_2–C_4) acyl groups between CoA and carnitine so that these acylcarnitines can cross intracellular membranes such as the mitochondrial and peroxisomal membranes. CAT activity has been found in the mitochondria, peroxisomes and endoplasmic reticulum in mammals and it is believed that CAT is responsible for maintaining the intracellular acylCoA:CoASH ratio as well as for detoxifying and excreting harmful acyl compounds in the form of acylcarnitines (Bieber, 1988).

Since the earliest study on the purified enzyme from pigeon muscle (Chase, 1967), much research has been conducted and reported on the kinetic and biochemical analysis of this enzyme (Ramsay *et al.*, 2001). Kinetic studies indicate that the reaction follows a rapid-equilibrium random-order mechanism in which all four substrates can bind to the free enzyme. On the other hand, active-site-directed chemical modification and site-directed mutagenesis studies identified an active-site histidine residue which is believed to serve as a general base/acid catalyst to facilitate the acyl-group transfer reaction (Ramsay *et al.*, 2001). Other active-site residues such as arginine and cysteine have also been reported to be involved in the reaction, although the precise mechanism remains unknown.

Additionally, CAT shows a significant amino-acid sequence similarity with a group of acyltransferases such as choline acetyltransferase (EC 2.3.1.6), carnitine octanyltransferase (EC 2.3.1.137) and carnitine palmitoyltransferases (EC 2.3.1.21). However, there is no three-dimensional structure available for any member of this group of acyltransferases. In order to understand the structural basis of catalysis and the reaction mechanism, knowledge of the detailed three-dimensional crystallographic structure of carnitine acetyltransferase is essential. To this end, we report here the crystallization and preliminary X-ray analysis of recombinant human CAT (hCAT).

2. Materials and methods

2.1. Purification

The expression and purification of the recombinant hCAT will be reported in detail elsewhere. Briefly, newly transformed bacterial cells harboring recombinant human CAT expression plasmid were grown at 303 K to an OD of 0.5 at 600 nm; 1 m*M* IPTG was then added and the cells induced for a further 2 h. Bacterial cells were then harvested by centrifugation and frozen at 193 K until further use. The purification procedure involves application of the *Escherichia coli* extract, prepared in 20 m*M* sodium phosphate buffer pH 7.6, onto a column of Ni-nitriloacetate-agarose (Qiagen) equilibrated with the loading buffer. The column is washed batchwise with buffer containing increasing concentrations of NaCl from 0 to 2 *M* and lastly with loading buffer containing 5 m*M* imidazole pH 7.4. The enzyme was eluted with an imidazole gradient from 5 to 150 m*M* and then applied onto a MonoQ column (Pharmacia) previously equilibrated with 20 m*M* sodium phosphate buffer pH 7.6. After washing the column with equilibration buffer, the enzyme was eluted with a linear salt gradient from 0 to 1 *M* and concentrated/dialyzed using Ultra-free Tangential membranes (Millipore). The puri-

fied enzyme was either used immediately or stored at 193 K in 20 m*M* sodium phosphate buffer pH 7.6 containing 40% glycerol.

2.2. Crystallization

Prior to crystallization trials, the purified recombinant hCAT was concentrated using Ultra-free Tangential membranes (Millipore) with a molecular-weight cutoff of 30 kDa. Initial crystallization experiments were carried out using the hanging-drop vapour-diffusion method in a 24-well tissue-culture Linbro plate (ICN Inc.) at three different temperatures: 277, 289 and 295 K. Each drop was formed by mixing equal volumes (3 µl) of 20 mg ml^{-1} enzyme solution and reservoir solution. A sparse-matrix crystallization screen based on the original report by Jancarik & Kim (1991) was used and promising conditions were further optimized with respect to pH and precipitants. The optimal condition was found to consist of 50 m*M* bis-Tris buffer pH 6.2, 100 m*M* NaCl and 12–15% PEG 8000 (Fig. 1).

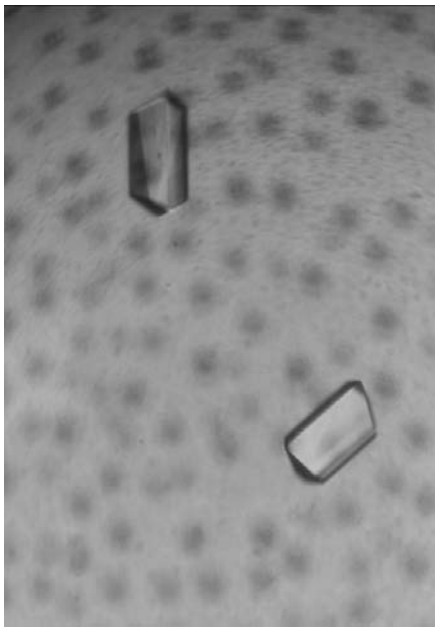

Figure 1
Photograph of recombinant human carnitine acetyltransferase crystals (approximate dimensions 0.4 × 0.2 × 0.2 mm).

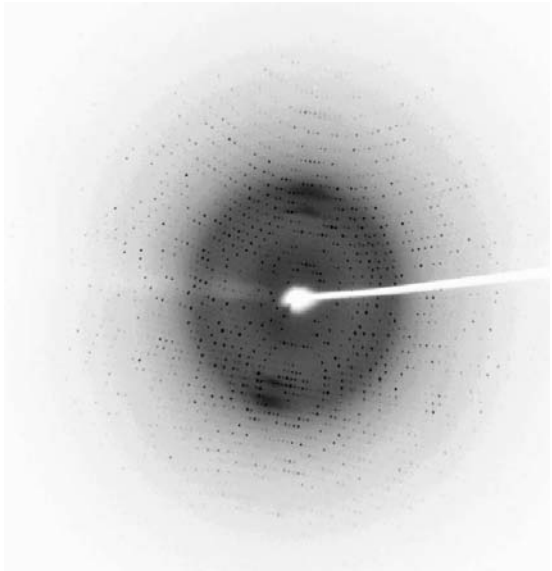

Figure 2
A typical oscillation photograph of human carnitine acetyltransferase crystal diffraction (highest resolution extended beyond 1.6 Å).

2.3. X-ray data collection and processing

X-ray intensity data collection was carried out using a Rigaku HU-H3R copper rotating-anode generator (equipped with Osmic mirrors) operating at 50 kV and 100 mA with Cu $K\alpha$ (λ = 1.5418 Å) radiation. X-ray diffraction intensity data were collected using an R-AXIS IV++ image-plate system. A single crystal of native hCAT was cryoprotected by a 60 s soak in a reservoir solution containing 15% PEG 8000 and 40% glycerol. The crystal was then suspended in a thin nylon loop and flash-frozen in the cryogenic (100 K) nitrogen stream prior to data collection. The diffraction data were collected using a crystal-to-detector distance of 150 mm with a 1° oscillation angle per image and a 300 s exposure time for each image using a 0.3 mm collimator. Fig. 2 shows a typical oscillation image, with diffraction data extending beyond 1.6 Å resolution. The diffraction data were integrated, scaled and reduced with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Table 1 describes the data-collection statistics of the hCAT crystal. The crystal has unit-cell parameters a = 137.70, b = 84.77, c = 57.87 Å and a scaling $R_{\rm sym}$ of 0.043 for 80 622 unique reflections (Table 2). The Laue symmetry and systematic absences are consistent with the $P2_12_12_1$ space group. The calculated Matthews coefficient (Matthews, 1968) indicates that one monomer of the protein is present in the asymmetric unit (2.77 Å^3 Da^{-1}) and this was also confirmed by self-rotation function calculation. Crystals of SeMet-CAT have now been obtained and it is expected that by using MAD data the structure determination of the protein will be obtained.

Table 1
Data-collection statistics for hCAT crystal.

Temperature (K)	100
Resolution (Å)	20–1.6
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 137.70, b = 84.77, c = 57.87
Wavelength (Å)	1.5418
Solvent content (%)	55
No. of molecules in asymmetric unit	1
Crystal dimensions (mm)	0.4 × 0.2 × 0.2
$V_{\rm M}$ (Å^3 Da^{-1})	2.77
Data collection	
No. of observations	724938
$I/\sigma(I)$	3.2

Table 2
Statistics of data collection from a hCAT crystal by resolution shells.

Resolution (Å)	No. unique reflections	Completeness (%)	$R_{\rm sym}$†
50.00–3.45	8658	92.2	0.022
3.45–2.74	8691	96.3	0.031
2.74–2.39	8665	96.7	0.048
2.39–2.17	8598	96.4	0.065
2.17–2.02	8452	95.2	0.089
2.02–1.90	8249	92.9	0.129
1.90–1.80	8032	91.1	0.192
1.80–1.72	7849	88.4	0.278
1.72–1.66	7514	85.2	0.363
1.66–1.60	5914	67.4	0.404
Total	80622	90.2	0.043

† $R_{\rm sym}$ is defined as $\sum|I - \langle I\rangle|/\sum\langle I\rangle$, where I is the intensity of an individual reflection and $\langle I\rangle$ is the average intensity for this reflection; the summation is over all intensities.

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