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Crystallization and preliminary X-ray crystallographic analysis of malonyl-CoA decarboxylase from *Rhizobium leguminosarum* bv. *trifolii*

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Malonyl-CoA decarboxylase (MCD), which catalyzes the conversion of malonyl-CoA to acetyl-CoA, is an evolutionarily distinct and highly conserved enzyme. MCD does not share sequence homology with other known decarboxylases, while the enzymes from different species exhibit at least >30% sequence identity to each other. In order to provide a canonical structure of the enzyme for detailed study of its structure–function relationship, the MCD of *Rhizobium leguminosarum* bv. *trifolii* was overexpressed and crystallized. The crystals belong to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 133.45$, $b = 127.10$, $c = 66.37$ Å. The asymmetric unit is likely to contain two molecules of MCD (molecular weight of 51 418 Da), with a crystal volume per protein weight (V_M) of 2.69 Å^3 Da^{-1} and a solvent content of about 54.3% by volume. A native data set to 3.0 Å resolution was obtained using a rotating-anode X-ray generator.

1. Introduction

Malonyl-CoA plays a pivotal role in fatty-acid metabolism. The metabolite is a precursor for fatty-acid biosynthesis as the donor of C_2 units (Wakil *et al.*, 1983). On the other hand, it is a physiological inhibitor of carnitine acyltransferases (McGarry *et al.*, 1989), which catalyze the esterification of carnitine and a long-chain fatty acid, the prerequisite step required for the β-oxidation of fatty acids in mitochondria or peroxisomes. Malonyl-CoA is biochemically synthesized by acetyl-CoA carboxylase, whereas it is hydrolyzed into acetyl-CoA and carbon dioxide by malonyl-CoA decarboxylase. Mutant mice lacking the gene coding for acetyl-CoA carboxylase II have a higher rate of fatty-acid oxidation, which is likely to be a consequence of lower levels of malonyl-CoA in the mitochondria compared with wild-type mice (Abu-Elheiga *et al.*, 2001).

The enzyme activity of malonyl-CoA decarboxylase (MCD; EC 4.1.1.9) has been detected in various organisms, including bacteria (An & Kim, 1998), goose (Kim & Kolattukudy, 1978*a*) and rat (Kim & Kolattukudy, 1978*b*). A significant role of MCD is suggested by severe phenotypic consequences arising from deficiency of the enzyme in humans, characterized by malonic aciduria, developmental delay, seizure disorder and mental retardation (Haan *et al.*, 1986; MacPhee *et al.*, 1993; Yano *et al.*, 1997). These phenotypes overlap with genetic deficiency of fatty-acid oxidation enzymes (Sacksteder *et al.*, 1999), indicating that MCD indeed plays critical roles in fatty-acid metabolism. MCD in vertebrates is found in different subcellular compartments (FitzPatrick *et al.*, 1999) and appears to have multiple functions depending on its location. MCD located in mitochondria may function in removing malonyl-CoA produced by the adventitious activity of propionyl-CoA carboxylase on acetyl-CoA. MCD located in cytoplasm may increase the synthesis of methyl branched-chain fatty acids by increasing the chance of incorporation of methylmalonyl-CoA rather than malonyl-CoA in the fatty-acid synthesis (Courchesne-Smith *et al.*, 1992). In non-adipogenic tissues such as cardiac and skeletal muscle, the cytoplasmic MCD enzyme could function in removing malonyl-CoA to allow fatty-acid oxidation to occur under the conditions where fatty acids are used as their primary energy source, *i.e.* exercised and/or fasted states (Sacksteder *et al.*, 1999). MCD located in the peroxisome, where β-oxidation of dicarboxylic fatty acids takes place, is proposed to eliminate malonyl-CoA, which is the end-product of the oxidation of odd chain-length dicarboxylic fatty acids (Sacksteder *et al.*, 1999).

A sequence alignment reveals that MCDs from different species exhibit at least greater than 30% sequence identity to each other and that MCDs share no detectable sequence homology with any other known decarboxylases, including methyl malonyl-CoA decarboxylases. Therefore, MCD is an evolutionarily distinct and highly conserved metabolic enzyme. In order to provide a canonical structure of MCDs and to begin understanding the catalytic mechanism of this enzyme, we initiated the structural study of MCD of

Rhizobium leguminosarum bv. *trifolii*. This bacterial MCD (accession code AAC83456) exhibits 30% sequence identity with human MCD (accession code XP_049311). In this report, we describe the overexpression and preliminary X-ray crystallographic analysis of this bacterial enzyme.

2. Protein expression and purification

The MCD gene was amplified by polymerase chain reaction from the bacterial genomic DNA using Pfu polymerase (Stratagene) and two primers (5′-GATGGATCCATGCGAGGCTTGGGGCCAAG-3′ and 5′-TGCTCAGAATTCTAGAGTTTGCTGTTG-3′). The PCR product was purified, digested with *Bam*HI and *Eco*RI and ligated into the pGEX-4T vector (Amersham-Pharmacia). The resulting vector was introduced into *Escherichia coli* BL21 (DE3) strain. The expression of the recombinant MCD fused to glutathione-*S*-transferase at the N-terminus was induced by 1 m*M* isopropyl-D-thiogalactopyranoside at an optical density of about 0.6 at 291 K. Bacterial cell lysate was prepared by sonication in buffer *A*, composed of 4.3 m*M* Na_2HPO_4, 137 m*M* NaCl, 1.27 m*M* KH_2PO_4 and 2.7 m*M* KCl. The supernatant fraction of the cell lysate was loaded onto a glutathione Sepharose 4B RediPack column (Amercham-Pharmacia). The fusion protein bound to the matrix was cleaved with thrombin (Roche Molecular Biomedicals) at a weight ratio of 20:1 (fusion protein: thrombin) for 18 h at 295 K. After centrifugation, the supernatant fraction was collected and loaded onto a HiLoad 26/60 Superdex 200 prep-grade column (Amercham-Pharmacia) pre-equilibrated with buffer *A*. The fractions containing MCD were concentrated using a Vivaspin 20 (Sartorius) in buffer *A* containing 1 m*M* β-mercaptoethanol. The final purified MCD (10 mg ml^{-1}) was at least 95% pure as judged on a polyacrylamide gel.

Table 1
Crystal information and data-collection statistics.

Values in parentheses are for the highest resolution shell, 3.1–3.0 Å.

Source	Rotating anode, Cu $K\alpha$
Wavelength (Å)	1.5418
Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 133.45, b = 127.10, c = 66.38
Resolution range (Å)	30–3.0
No. of unique reflections	21112
Data completeness (>1σ) (%)	90.5 (78.4)
R_{sym}† (%)	9.4 (34.0)
$I/\sigma(I)$	10.6 (1.1)

† $R_{sym} = \sum|I_{obs} - I_{avg}|/I_{obs}$, where I_{obs} is the observed intensity of individual reflections and I_{avg} is the average over symmetry equivalents.

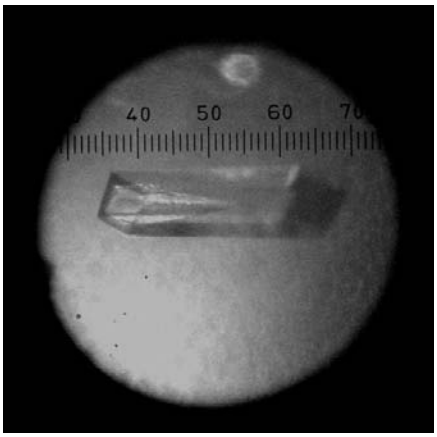

Figure 1
Crystals of MCD. Usually, the crystals have a hole at one end. Eight divisions on the ruler indicate 0.1 mm.

3. Crystallization of MCD

Crystals of MCD were obtained by the hanging-drop vapour-diffusion method at 298 K using 24-well Linbro plates (Hampton Research). A hanging drop was prepared by mixing equal volumes (1.5 µl each) of protein solution and reservoir solution. Each hanging drop was placed over 0.5 ml reservoir solution. Initial crystals were obtained with one of the solutions (#18) from the crystallization screening kit Wizard II (Emerald Biostructures). By varying the original crystallization conditions, large shiny well shaped crystals were obtained with precipitant solution *A* containing 13%(*w*/*v*) polyethylene glycol 3000, 0.1 *M* sodium acetate pH 4.5 and 0.2 *M* calcium acetate. However, these crystals were quite brittle and only diffracted X-ray to ∼8 Å resolution. Further variations of the crystallization conditions using Additive Screens (Hampton Research) led us to obtain much better crystals (Fig. 1) with precipitant solution *A* containing 20 m*M* L-cysteine.

4. Data collection and analysis

Diffraction data for MCD were collected with a crystal mounted in a capillary tube at room temperature on a Rigaku R-AXIS IV++ area-detector system with Cu $K\alpha$ X-rays generated by a rotating-anode generator operated at 100 mA and 50 kV. Diffraction data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The mosaicity of the crystals was typically about 0.3°. Because of the radiation-induced decay of the crystals, data sets collected from three different crystals were scaled and merged together (Table 1). The crystals belong to the orthorhombic space group $P2_12_12$. We calculated that the crystal volume per molecular weight (V_M) is 2.69 Å^3 Da^{-1}, with a solvent content of 54.3% by volume (Matthews, 1968) when one unit cell was assumed to contain eight molecules of MCD. This corresponds to two molecules of MCD per asymmetric unit. We have not yet found a suitable cryoprotectant for the crystals. We have so far tried to use glycerol, polyethylene glycol 400 and Paratone-N (Hampton Research), in which the MCD crystals were briefly immersed. All of these treatments damaged crystals as judged from peak shapes. The diffraction intensity is very strong at low resolution, but decreases rapidly at higher resolution such that a prolonged X-ray exposure of the crystals barely produces diffraction spots beyond 3.0 Å resolution. Refinement of the crystallization conditions for obtaining well diffracting crystals and a search for a suitable cryoprotectant is in progress in parallel with efforts to obtain crystals of selenomethionine-substituted protein.

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