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Crystallization and preliminary X-ray analysis of the 12S central subunit of transcarboxylase from *Propionibacterium shermanii*

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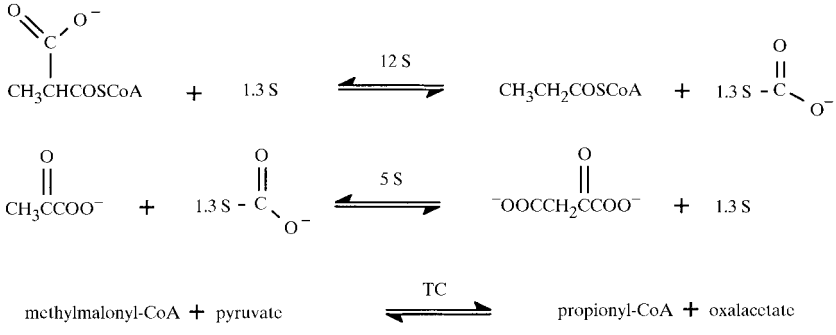
The hexameric 12S central subunit of transcarboxylase has been crystallized in both free and substrate-bound forms. The apo crystals belong to the cubic space group $P4_232$, with unit-cell parameters $a = b = c = 188.5$ Å, and diffract to 3.5 Å resolution. Crystals of two substrate-bound complexes, 12S with methylmalonyl CoA and 12S with malonyl CoA, are isomorphous and belong to space group $C2$, with unit-cell parameters $a = 115.5$, $b = 201.4$, $c = 146.9$ Å, $\beta = 102.7^\circ$. These crystals diffract to 1.9 Å resolution with synchrotron radiation. Two useful heavy-atom phasing derivatives of methylmalonyl CoA-bound crystals have been obtained by co-crystallization or crystal soaking.

1. Introduction

Transcarboxylase (TC) from *P. shermanii* is a 1200 kDa multisubunit complex composed of 30 polypeptides of three types: a central hexameric 12S subunit, six dimeric 5S subunits and 12 1.3S biotinylated linkers (reviewed in Wood & Zwolinski, 1976; Wood, 1979; Wood & Kumar, 1985). According to electron microscopy, TC has a cylindrical appearance with a cavity in the center; three dimeric 5S subunits form a ring at each end of the 12S core in the multienzyme complex (Wood & Kumar, 1985). 12 biotinylated 1.3S subunits link the six dimeric 5S subunits to the central 12S. The 12S subunit consists of six 605-residue 66 kDa monomers (Thornton, Kumar, Haase *et al.*, 1993). The 5S subunit is a homodimer of a metalloprotein containing Co^{2+} and Zn^{2+} and a 519-residue 58 kDa polypeptide chain (Thornton, Kumar, Shenoy *et al.*, 1993). The 123-residue 12 kDa 1.3S subunit has been investigated by solution NMR (Reddy *et al.*, 1997, 1998, 2000), but no detailed structural information is currently available for either the 12S or 5S subunits.



Transcarboxylase catalyzes the reversible transfer of a carboxylate group from methylmalonyl CoA to pyruvate, generating propionyl CoA and oxalacetate. The transcarboxylation reaction is carried out in two steps, catalyzed by the 12S and 5S subunits.

This transcarboxylation, in contrast to other biotin enzyme-catalyzed reactions, does not involve free CO_2, HCO_3^- or ATP. However, individual steps in the transcarboxylase catalyzed carboxylate-transfer reaction are similar to those carried out by a variety of biologically important carboxylases such as the biotin-dependent carboxylases in mammals. These enzymes catalyze key steps in several fundamental metabolic pathways, including fatty-acid biosynthesis, gluconeogenesis and amino-acid catabolism. Historically, TC has served as a model system for the mammalian carboxylases. The 12S subunit is most similar to the α-chains of methymalonyl CoA decarboxylase and the β-chains of propionyl CoA carboxylase, which catalyze an identical reaction, and the β-chains of acetyl CoA carboxylase. As there is very limited structural information available for these (de)carboxylases, elucidation of the 12S subunit crystal structure may provide mechanistic insight not only into the TC reaction but also into other related carboxyl transfers.

2. Material and methods

2.1. Expression and purification

A C-terminally truncated 12S subunit (524 residues compared with 609 in the full-length chain) was expressed and purified as described previously (Woo *et al.*, 1993). A plasmid encoding the truncated 12S subunit was transformed into *Escherichia coli* strain JM109. The cells were grown in 2 × YT medium at 310 K overnight. Expression was induced by 1:100 dilution of the starter into 2 × YT medium containing 1 m*M* IPTG. After 10 h of

Table 1
Data-processing statistics.

Values in parentheses are for the highest resolution shell.

Crystals	Apo-12S	MMCoA	MMCoA–Cd	MMCoA–Cd–Hg	MMCoA–Cd–Ag	MCoA–Cd
X-ray source	In-house	APS	APS	In-house	ALS	In-house
Space group	$P4_232$	$C2$	$C2$	$C2$	$C2$	$C2$
Unit-cell parameters						
a (Å)	189.68	115.5	115.3	115.2	290.6	114.9
b (Å)		201.4	201.4	201.1	201.0	200.3
c (Å)		146.9	146.5	146.5	115.0	145.5
β (°)		102.7	102.5	102.4	99.96	102.4
V_M (Å^3 Da^{-1})	2.53 or 1.68	2.47	2.46	2.45	2.45	2.42
Monomers per asymmetric unit	2 or 3	6	6	6	12	6
Resolution (Å)	100.0–3.5 (3.63–3.5)	30.0–2.1 (2.18–2.1)	30.0–1.9 (1.97–1.9)	100.0–2.8 (2.9–2.8)	25.0–2.2 (2.28–2.2)	100.0–2.4 (2.49–2.4)
No. of observations	65658	765615	1061589	262593	1083910	157362
Unique reflections	11377 (652)	172774 (11119)	234797 (17439)	74725 (6933)	321529 (28670)	96478 (7675)
Mean $I/\sigma(I)$	12.9 (7.8)	16.0 (1.9)	20.0 (4.9)	22.3 (5.2)	10.6 (2.4)	13.2 (5.8)
R_{sym} (%)	6.2 (20.7)	6.5 (34.7)	5.5 (23.6)	7.3 (18.7)	7.2 (30.1)	4.3 (10.9)
Completeness (%)	74.2 (43.2)	90.2 (58.2)	92.6 (69.1)	93.4 (86.7)	98.1 (87.8)	77.2 (61.8)

accumulated expression, cells were harvested by centrifugation. About 30 g of cells (from 6 l of culture) were suspended in 90 ml of 25 m*M* potassium phosphate buffer pH 7.2 containing 1 m*M* DTT and 0.1 m*M* each of EDTA, PMSF and sodium azide (buffer *A*) and then lysed by passage through a French press twice at 13.8 MPa. The lysate was centrifuged at 27 000*g* for 30 min (all the subsequent experimental steps were performed at 277 K) and the nucleic acid was removed by precipitation and centrifugation after streptomycin sulfate addition to the supernatant to a final concentration of 10%(*w*/*v*). The resulting supernatant was fractionated with 45% ammonium sulfate in buffer *A*; after centrifugation, the pellet was resuspended in buffer *A* and then dialyzed against buffer *A* overnight.

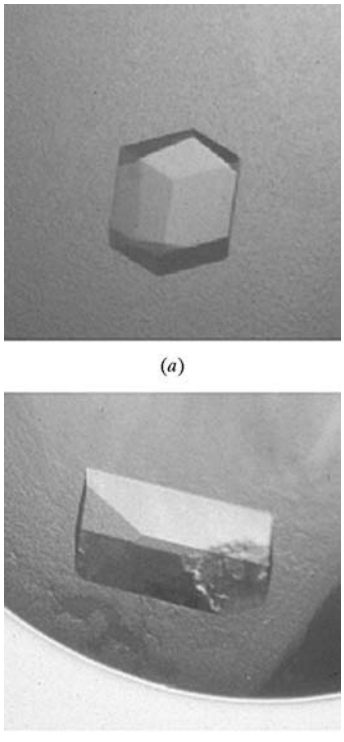


Figure 1
(*a*) A crystal of apo 12S, typically measuring up to ~0.5 mm; (*b*) a crystal of 12S–MMCOA, typically measuring up to 0.7 mm.

The 12S subunit solution was purified through Whatman DE-52 and Whatman P-11 ion-exchange columns and a Bio-gel 1.5 m size-exclusion column. The dialysate was first loaded onto the pre-equilibrated DE-52 column. After extensively washing with buffer *A*, elution was carried out with different concentrations of potassium phosphate (100, 200 and 500 m*M*). The 12S-containing fractions usually eluted in 500 m*M* potassium phosphate and were concentrated and dialyzed against 25 m*M* potassium phosphate buffer pH 6.5 containing 1 m*M* DTT and 0.1 m*M* each of EDTA, PMSF and sodium azide (buffer *B*). The dialysate was loaded to a P-11 column, washed with buffer *B* and eluted with three concentration steps of potassium phosphate as described above. Finally, 12S was passed through the size-exclusion column in 250 m*M* potassium phosphate buffer pH 6.5 containing 1 m*M* DTT and 0.1 m*M* each of EDTA, PMSF and sodium azide (buffer *C*). Typically, 6–8 mg of high-purity 12S can be purified from 30 g of cells.

2.2. Crystallization

Prior to crystallization, the purified 12S was dialyzed against 20 m*M* potassium phosphate buffer at pH 6.0 containing 1 m*M* DTT and then concentrated to about 25 mg ml^{-1}. For complex formation, the concentrated 12S was incubated either with 5 m*M* methylmalonyl CoA (MMCoA) or 5 m*M* malonyl CoA (MCoA, an analog of MMCoA that is utilized as efficiently as the natural substrate) overnight at 277 K. Crystallization was carried out at 293 K using the sitting-drop vapor-diffusion method. Initial conditions were based on those reported earlier for substrate-free 12S (Skrzypczak-Jankun *et al.*, 1986) as well as using the sparse-matrix method (Jancarik & Kim, 1991; Hampton Research, California, USA).

12S apo crystals (*i.e.* in the absence of ligand) were grown from 0.1 *M* HEPES pH 7.5 and 30–31% 2,4-methylpentanediol (MPD), similar to the reported conditions. These crystals are cubic and diffract poorly, as also reported previously (Skrzypczak-Jankun *et al.*, 1986). A new crystal form was found for both the 12S–MMCoA and 12S–MCoA complexes. These crystals were grown from 0.1 *M* sodium acetate pH 4.5 and 20–25% MPD and diffract to up to 2.1 Å.

Crystals of 12S–MMCoA and 12S–MCoA co-crystallized with 5 m*M* $CdCl_2$ survived longer and diffracted to higher resolution (up to 1.9 Å); the usefulness of cadmium as an additive has been observed with other proteins (Trakhanv & Quiocho, 1995; Trakhanv *et al.*, 1998). These crystals are isomorphous to each other and to the complex crystals grown in the absence of cadmium. Since the cadmium-containing crystals grew more reproducibly and were more stable, they were used as 'native' crystals for heavy-metal soaks. Even then, these crystals are not easily amenable to soaking in artificial mother-liquor solutions, so mercury acetate and silver nitrate derivatives could be obtained only by adding the heavy-metal solution directly to the crystallization drop.

2.3. Data collection and data processing

Initial data sets were collected using an in-house Rigaku rotating-anode generator operating at 50 kV and 100 mA and equipped with an R-AXIS IV imaging-plate detector and Yale mirrors. Higher resolution and more complete complex data sets were collected with synchrotron-radiation sources (ALS 5.0.2, APS 19-ID and NSLS X12B). All data sets were measured using crystals cooled to 93 K. Fortunately, crystals could be mounted directly from the crystallization drop as the mother liquor was an effective cryoprotectant. The data sets were processed with *HKL* (Otwinowski & Minor, 1997) and are summarized in Table 1.

3. Results and discussion

The apo 12S was crystallized under conditions similar to those reported previously (Skrzypczak-Jankun *et al.*, 1986); the crystals are fragile (Fig. 1*a*). The crystals contain protein C-terminally truncated at residue 524, rather than the full-length protein in the previously described crystals. This may explain the difference in diffraction behavior. Our apo 12S crystals diffract to only 3.5 Å resolution and did not improve with synchrotron-radiation sources. In biochemical studies, C-terminally truncated 12S mutants that contain more than 508 residues still form active hexamers (Woo *et al.*, 1993). Our crystals indicate that truncation may alter crystal packing and affect diffraction behavior.

The 12S–MMCoA and 12S–MCoA complex crystals were observed about 3 d after the trays were set up and continued to grow for up to two weeks (Fig. 1*b*). These crystals can survive for more than two months in the crystallization trays with no deterioration in diffraction behavior. The crystals of the substrate complexes are a new crystal form with space group *C*2. These crystals are sturdier, easier to manipulate and diffract to higher resolution than the apo crystals. Complex crystals co-crystallized with cadmium are improved further. The best 12S data are from a 12S–MMCoA complex co-crystallized with cadmium and extend to 1.9 Å resolution with 92.6% completeness.

Data for 12S–MMCoA were first measured using an in-house source. An 83.9% complete data set to 2.8 Å resolution was collected. Assuming one 12S hexamer in the asymmetric unit and with a truncated molecular weight of 338 000 Da, the Matthews coefficient V_M (Matthews, 1968) was calculated to be 2.47 Å^3 Da^{-1}, corresponding to 50.1% solvent content. An isomorphous 12S–MCoA data set was also collected to 2.4 Å resolution. The crystals co-crystallized with 5 m*M* $CdCl_2$ diffract much better and are more stable during the data collection. In-house data for 12S–MMCoA–Cd were collected to 2.2 Å resolution. The 12S–MMCoA–Cd crystals were used as 'native' and then soaked with 1 m*M* mercury acetate for derivatization; data were measured to 2.8 Å resolution. Both derivative data sets were isomorphous to that of the 12S–MMCoA.

With the APS synchrotron source, 12S–MMCoA and 12S–MMCoA–Cd crystals diffracted to much higher resolutions: 2.1 Å and 1.9 Å, respectively. The data sets are more complete and isomorphous to the data sets collected in-house. Also, data from a crystal of 12S–MMCoA–Cd soaked with $AgNO_3$ were measured to 2.2 Å resolution at ALS. Unexpectedly, derivatization altered the crystal packing and doubled one unit-cell parameter; the new crystal form is *C*2 with a = 290.64, b = 201.00, c = 115.00 Å, β = 99.96°.

The structure of 12S–MMCoA is currently being solved by multiple isomorphous replacement using both Cd and Hg derivatives. Three Cd and 12 Hg sites have been located by difference Patterson and cross-difference Fourier methods and allowed identification of non-crystallographic threefold and twofold axes. After phasing with *SHARP* (de La Fortelle & Bricogne, 1997) and non-crystallographic averaging and solvent flattening in *DM* (Collaborative Computational Project, Number 4, 1994) exploiting the 32 symmetry identified by inspection of the heavy-atom sites and the MIR density, modified density maps could be interpreted for model building. Completion of the 12S–MMCoA structure is currently under way.

After the 12S complex structure is determined, the structure of apo 12S will be solved by molecular replacement. Unfortunately, the current diffraction data set for the apo 12S crystal form is not of sufficient quality, completeness or resolution to provide a clear molecular-replacement solution. The most likely calculated V_M is 2.53 Å^3 Da^{-1} (two molecules per asymmetric unit, 51.3% solvent), although a V_M of 1.68 Å^3 Da^{-1} is also possible (three molecules per asymmetric unit, 27.0% solvent); both would be consistent with a 12S hexamer of 32 symmetry placed on a crystallographic axis. For the determination of this structure, we are continuing to optimize the diffraction of apo 12S crystals by crystallizing different C-terminally truncated constructs and full-length protein.

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References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Jancarik, J. & Kim, S.-H.(1991). *J. Appl. Cryst.* **24**, 409–411.
La Fortelle, E. de & Bricogne, G. (1997). *Methods Enzymol.* **276**, 472–494.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 306–326.
Reddy, D. V., Rothemund, S., Shenoy, B. C., Carey, P. R. & Sonnichsen, F. D. (1998). *Protein Sci.* **7**, 2156–2163.
Reddy, D. V., Shenoy, B. C., Carey, P. R. & Sonnichsen, F. D. (1997). *Biochemistry*, **36**, 14676–14682.
Reddy, D. V., Shenoy, B. C., Carey, P. R. & Sonnichsen, F. D. (2000). *Biochemistry*, **39**, 2509–2516.
Skrzypczak-Jankun, E., Tulinsky, A., Fillers, J. P., Kumar, K. G. & Wood, H. G. (1986). *J. Mol. Biol.* **188**, 495–498.
Thornton, C. G., Kumar, G. K., Haase, F. C., Phillips, N. F., Woo, S. B., Park, V. M., Magner, W. J., Shenoy, B. C., Wood, H. G. & Samols, D. (1993). *J. Bacteriol.* **175**, 5301–5308.
Thornton, C. G., Kumar, G. K., Shenoy, B. C., Haase, F. C., Phillips, N. F., Park, V. M., Magner, W. J., Hejlik, D. P., Wood, H. G. & Samols, D (1993). *FEBS Lett.* **330**, 191–196.
Trakhanv, S., Kreimer, D. I., Parkin, S., Ames, G. F. & Rupp, B. (1998). *Protein Sci.* **7**(3), 600–604.
Trakhanv, S. & Quiocho, F. A. (1995). *Protein Sci.* **4**, 1914–1919.
Woo, S. B., Shenoy, B. C., Wood, H. G., Magner, W. J., Kumar, G. K., Beegen, H. & Samols, D. (1993). *J. Biol. Chem.* **268**, 16413–16419.
Wood, H. G. (1979). *CRC Crit. Rev. Biochem.* **7**, 143–160.
Wood, H. G. & Kumar, G. K. (1985). *Ann. NY Acad. Sci.* **447**, 1–21.
Wood, H. G. & Zwolinski, G. K. (1976). *CRC Crit. Rev. Biochem.* **4**, 47–122.