Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Cubic crystals of phosphopantetheine adenylyltransferase from *Escherichia coli*

Tina Izard,[a]* Arie Geerlof,[b] Ann Lewendon[b] and John J. Barker[b]

[a]Department of Biochemistry, University of Leicester, Leicester LE1 7RH, England, and [b]Department of Microbiology and Immunology, University of Leicester, Leicester LE1 9HN, England

Correspondence e-mail: ti3@le.ac.uk

Received 29 December 1998
Accepted 24 March 1999

Phosphopantetheine adenylyltransferase (PPAT, E.C. 2.7.7.3) catalyzes the penultimate step in coenzyme A (CoA) biosynthesis, transferring an adenylyl group from ATP to 4′-phosphopantetheine, and forming dephospho-CoA. Cubic crystals of native PPAT from *Escherichia coli* as well as PPAT in complex with its substrates were obtained. The crystals belong to space group $I23$ or $I2_13$ with unit-cell dimension a = 135.5 Å. The crystals diffract to better than 1.8 Å resolution on a Cu $K\alpha$ rotating-anode generator. The asymmetric unit is likely to contain two molecules, corresponding to a packing density of 2.9 Å^3 Da^{-1}.

1. Introduction

Coenzyme A (CoA), the principal acyl-group carrier in all living cells, is required for numerous reactions in intermediary metabolism (Robinshaw & Neely, 1985). CoA is synthesized from pantothenate (vitamin B_5), cysteine and ATP in five steps. The penultimate step is the transfer of an adenylyl group from ATP to 4′-phosphopantetheine, catalyzed by phosphopantetheine adenylyltransferase (PPAT) and yielding dephospho-CoA (dPCoA) and pyrophosphate. Subsequent phosphorylation at the 3′-hydroxyl of the ribose ring by dephospho-CoA kinase (dPCoAK) produces the acyl-group carrier, CoA.

Although the rate of CoA biosynthesis is believed to be regulated by feedback inhibition of pantothenate kinase (PtK), the enzyme which catalyzes the first step in the CoA biosynthetic pathway (Halvorsen & Skrede, 1982; Fisher & Neely, 1985; Vallari *et al.*, 1987; Falk & Guerra, 1993), the fact that pantothenate and 4′-phosphopantetheine accumulate in the cell (Vallari & Rock, 1984) indicates that PPAT might also play a role in controlling the rate of CoA biosynthesis.

In higher organisms, PPAT and dPCoAK occur as a bifunctional enzyme complex (Suzuki *et al.*, 1967; Worrall & Tubbs, 1983), leading to the common use of the term 'CoA synthase' to describe these two activities. CoA synthase from pig liver has been reported to be a dimer of identical subunits of M_r 57 kDa (Worrall *et al.*, 1985), and limited proteolysis suggested that each subunit contains both activities. Similar bifunctional enzyme complexes have been reported from other mammalian sources (Stewart *et al.*, 1968; Suzuki *et al.*, 1967). In bacteria, however, PPAT and dPCoAK occur as separate enzymes. PPAT from *Brevibacterium ammoniagenes* has been characterized and used for synthesis of CoA analogues (Martin & Drueckhammer, 1993). The gene encoding PPAT from *Echerichia coli* has recently been cloned (Geerlof *et al.*, 1999). Recombinant PPAT has been overexpressed, purified and shown to have PPAT activity (Geerlof *et al.*, 1999). The polypeptide chain has a molecular weight of 17.8 kDa. PPAT shows no significant sequence identity to proteins whose structure is known. A search for heavy-atom derivatives in order to solve the structure *via* isomorphous replacement has been initiated.

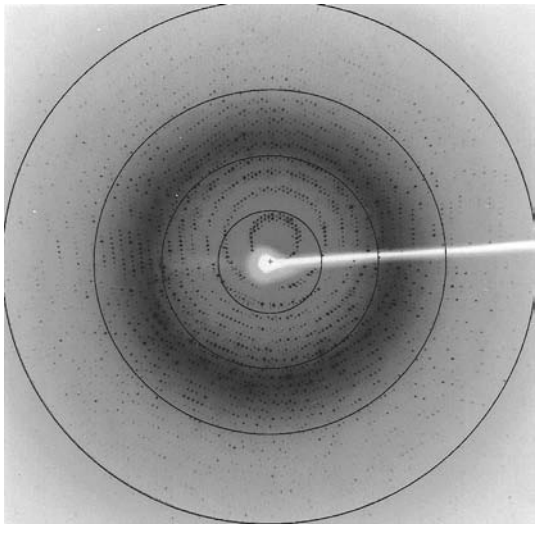

Figure 1
A typical diffraction pattern of PPAT crystals. This image was recorded from PPAT in complex with ATP on an R-AXIS IV imaging plate with a crystal-to-detector distance of 150 mm and an exposure time of 20 min. The oscillation range of this image is 0.5°. The resolution rings are at 8.1, 4.0, 2.7 and 2.0 Å.

Table 1
Crystallization conditions for various complexes of PPAT.

AMPCPP: α,β-methyleneadenosine 5′-triphosphate. Ppant: 4′-phosphopantetheine. The ternary complex crystals were obtained by either soaking the PPAT–AMPCPP crystals with 4′-phosphopantetheine or by soaking the PPAT–Ppant crystals with AMPCPP. All other crystals were co-crystallized.

	Apo form	PPAT–CoA	PPAT–AMPCPP–Ppant	PPAT–Ppant	PPAT–AMPCPP	PPAT–ATP	PPAT–dPCoA
$(NH_4)_2SO_4$ (*M*)	1.2	1.1	1.1	1.4	1.1	1.2–1.4	1.1
Sodium chloride (*M*)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.1 *M* sodium acetate (pH)	4.9	5.0	5.0	4.9	5.0	4.7–4.9	5.0
Ligands (10 m*M*)	—	CoA	AMPCPP, Ppant	Ppant	AMPCPP	ATP	dPCoA
Additives (10 m*M*)	—	—	$MnCl_6$	—	$MnCl_6$	$MnCl_6$	—

Table 2
Data-reduction statistics of various PPAT data sets.

AMPCPP: α,β-methyleneadenosine 5′-triphosphate. Ppant: 4′-phosphopantetheine. PPAT–CoA and PPAT–AMPCPP–Ppant data sets were collected at Brookhaven National Laboratory synchrotron facility with a wavelength of 1.1 Å. All other data sets were collected using a Cu *Kα* rotating-anode source with an R-AXIS IV imaging plate.

	Apo form	PPAT–CoA	PPAT–AMPCPP–Ppant	PPAT–Ppant	PPAT–AMPCPP	PPAT–ATP	PPAT–dPCoA
Total data	791888	361315	380523	522214	803931	734495	390875
Unique data	37459	37147	38276	37875	37463	37486	37504
Redundancy	21.1	9.73	9.94	13.79	21.45	19.60	10.42
$F^2 > 3\sigma(F^2)$ (100–1.8 Å) (%)	78.5	86.4	92.0	94.8	88.6	86.6	89.7
$F^2 > 3\sigma(F^2)$ (1.86–1.8 Å) (%)	78.5	86.4	92.0	94.8	88.6	87.3	89.7
Average $F^2/\sigma(F^2)$	30.7	24.1	23.4	30.8	32.5	39.4	37.8

$R_{\rm merge}$†

Resolution range (Å)	Apo form	PPAT–CoA	PPAT–AMPCPP–Ppant	PPAT–Ppant	PPAT–AMPCPP	PPAT–ATP	PPAT–dPCoA
100.00–3.88	0.026	0.037	0.049	0.041	0.025	0.026	0.019
3.88–3.08	0.037	0.024	0.040	0.058	0.031	0.033	0.023
3.08–2.69	0.052	0.030	0.065	0.075	0.040	0.042	0.030
2.69–2.44	0.075	0.034	0.074	0.086	0.051	0.053	0.037
2.44–2.27	0.108	0.039	0.335	0.094	0.063	0.062	0.046
2.27–2.13	0.135	0.048	0.415	0.105	0.077	0.077	0.057
2.13–2.03	0.183	0.057	0.462	0.116	0.104	0.101	0.074
2.03–1.94	0.224	0.078	0.217	0.128	0.126	0.121	0.091
1.94–1.86	0.304	0.115	0.239	0.142	0.162	0.148	0.113
1.86–1.80	0.448	0.144	0.305	0.157	0.223	0.195	0.141
100.00–1.80	0.043	0.036	0.158	0.062	0.039	0.038	0.028

Completeness (%)

Resolution range (Å)	Apo form	PPAT–CoA	PPAT–AMPCPP–Ppant	PPAT–Ppant	PPAT–AMPCPP	PPAT–ATP	PPAT–dPCoA
100.00–3.88	99.9	96.3	98.2	99.5	99.7	99.0	98.9
3.88–3.08	100.0	98.5	99.7	100.0	100.0	99.8	100.0
3.08–2.69	100.0	97.9	99.9	100.0	100.0	99.9	100.0
2.69–2.44	100.0	97.8	99.9	100.0	100.0	100.0	100.0
2.44–2.27	100.0	97.0	100.0	100.0	100.0	100.0	100.0
2.27–2.13	100.0	97.2	100.0	100.0	100.0	99.9	100.0
2.13–2.03	100.0	97.4	100.0	99.9	100.0	100.0	100.0
2.03–1.94	99.9	97.2	100.0	99.4	99.3	98.8	99.4
1.94–1.86	96.3	95.5	100.0	96.4	93.1	95.3	94.2
1.86–1.80	80.0	94.4	99.9	87.1	84.2	83.6	82.5
100.00–1.80	97.4	96.9	99.8	98.2	97.6	97.7	97.5

† $R_{\rm merge} = \sum_{\rm unique\ reflections}(\sum_{i=1}^{N}|I_i - \bar{I}|)/\sum_{\rm unique\ reflections}(\sum_{i=1}^{N}|I_i|)$.

2. Methods and results

PPAT was purified to homogeneity by column chromatography as described elsewhere (Geerlof *et al.*, 1999). Crystallization experiments were performed at room temperature using the hanging-drop vapour-diffusion technique.

The purified enzyme was dialysed into 10 m*M* HEPES buffer pH 8 containing 0.5 m*M* DTT and concentrated to 18 mg ml^{-1}. Crystals of native PPAT were obtained from 1.1 *M* $(NH_4)_2SO_4$, 0.2 *M* sodium chloride and 100 m*M* sodium acetate (pH 5). Initial crystallization droplets of 3 µl resulted in PPAT crystals of dimensions up to 0.1 mm. Increasing the drop size to 16 µl resulted in PPAT crystals up to 1 mm in each dimension within two weeks.

Co-crystals of PPAT in complex with ligands were grown under similar conditions as the apo form, but including 10 m*M* ligand in the crystallization drop. All crystals grew within two weeks to dimensions up to 1 mm. Crystals of PPAT–4′-phosphopantetheine binary complex showed slightly different growth behaviour in that many small crystals appeared within one week, some of which displayed different morphology, such as two-dimensional plates. Crystals of a non-catalytic ternary complex of PPAT with the non-hydrolyzable ATP analogue, α,β-methyleneadenosine 5′-triphosphate (AMPCPP) and 4′-phosphopantetheine (PPAT–AMPCPP–4′-phosphopantetheine) were obtained either by soaking PPAT–AMPCPP co-crystals with 4′-phosphopantetheine or by soaking PPAT–4′-phosphopantetheine crystals with AMPCPP. No ternary complex crystals were obtained from protein plus the two ligands. All crystals were cryo-protected by including 35% glycerol in the mother liquor. Crystallization conditions are summarized in Table 1.

X-ray data of the apo form and PPAT co-crystals (PPAT–4′-phosphopantetheine, PPAT–AMPCPP, PPAT–ATP and PPAT–dPCoA) were collected at 100 K (Fig. 1) using a Cu *Kα* rotating-anode source with an R-AXIS IV imaging plate. The data were collected at a crystal-to-detector distance of 150 mm using 0.5° oscillations per image and an exposure time of 20 min per frame.

X-ray data of the ternary complex (PPAT–AMPCPP–4′-phosphopantetheine) and of PPAT in complex with its inhibitor CoA were collected at beamline X12-C of the National Synchrotron Light Source, Brookhaven National Laboratory. A Brandeis CCD detector was used to record the images. Frozen crystals (100 K) showed strong diffraction to 1.6 Å resolution. The X-ray data were collected at a crystal-to-detector distance of 225 mm using 0.5° oscillations per image. The wavelength and exposure time were 1.1 Å and 1 min, respectively.

Autoindexing with *DENZO* determined that the crystals belong to the cubic body-centered crystal system with unit-cell dimension a = 135.5 Å. Analysis of the simulated 0*kl* (or *hk*0 or *h*0*l*) precession photographs showed only *mm* symmetry and no fourfold symmetry. Thus, the crystals belong to space group *I*23 (or its enantiomer $I2_13$). An assumption of two protomers per asymmetric unit leads to an acceptable

packing density, V_m (Matthews, 1968), of 2.9 Å^3 Da^{-1}, corresponding to a solvent content of about 57%. All data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Data statistics are given in Table 2.

We thank Bill Shaw (Leicester) for critical reading of the manuscript. Some diffraction data for this study were collected at Brookhaven National Laboratory using the Biology Department single-crystal diffraction facility at beamline X12-C at the National Synchrotron Light Source. This facility is supported by the United States Department of Energy Offices of Health and Environmental Research and of Basic Energy Sciences, and by the National Science Foundation. We thank Bob Sweet (BNL) for his invaluable assistance during data collection. We are grateful to Bob Liddington (Leicester) for his continuous support and Peter Moody (Leicester) for fruitful discussion. TI acknowledges a Wellcome Trust Travel Grant. AG is supported by a Wellcome Trust project grant (046311/Z/95) awarded to W. V. Shaw.

References

Falk, K. L. & Guerra, D. J. (1993). *Arch. Biochem. Biophys.* **301**, 424–430.
Fisher, M. N. & Neely, J. R. (1985). *FEBS Lett.* **190**, 293–296.
Geerlof, A., Lewendon, A. & Shaw, W. V. (1999). In the press.
Halvorsen, O. & Skrede, S. (1982). *Eur. J. Biochem.* **124**, 211–215.
Martin, D. P. & Drueckhammer, D. G. (1993). *Biochem. Biophys. Res. Commun.* **192**, 1155–1161.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Robinshaw, J. D. & Neely, J. R. (1985). *Am. J. Physiol.* **248**, E1–E9.
Stewart, C. J., Thomas, J. O., Ball, W. J. Jr & Aguirre, A. R. (1968). *J. Am. Chem. Soc.* **28**, 5000–5004.
Suzuki, T., Abiko, Y. & Shimizu, M. (1967). *J. Biochem.* **62**, 542–649.
Vallari, D. S., Jackowski, S. & Rock, C. O. (1987). *J. Biol. Chem.* **262**, 2468–2471.
Vallari, D. S. & Rock, C. O. (1984). *J. Bacteriol.* **158**, 115–120.
Worrall, D. M., Lambert, S. F. & Tubbs, P. K. (1985). *FEBS Lett.* **187**, 277–279.
Worrall, D. M. & Tubbs, P. K. (1983). *Biochem. J.* **215**, 153–157.