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Preliminary X-ray diffraction analysis of octaprenyl pyrophosphate synthase crystals from *Thermotoga maritima* and *Escherichia coli*

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Octaprenyl pyrophosphate synthase (OPPs) catalyzes the condensation of five isopentenyl pyrophosphates with farnesyl pyrophosphate to generate C_{40} octaprenyl pyrophosphate. The enzymes from the hyperthermophilic bacterium *Thermotoga maritima* and from the mesophilic *Escherichia coli* were expressed in *E. coli* and the recombinant proteins were purified and crystallized. The *T. maritima* OPPs crystals belong to space group $P42_12$, with unit-cell parameters $a = b = 151.53$, $c = 69.72$ Å. The *E. coli* OPPs crystals belong to space group $C222_1$, with unit-cell parameters $a = 247.66$, $b = 266.10$, $c = 157.93$ Å. Diffraction data were collected at 100 K using synchrotron radiation and an in-house X-ray source. Structure determination of *T. maritima* OPPs has been carried out using MIR data sets at 2.8 Å resolution. The asymmetric unit contains one dimer. An initial model with 280 residues per subunit has been built and refined to 2.28 Å resolution. It shows mostly helical structure and resembles that of avian farnesyl pyrophosphate synthase.

1. Introduction

Prenyltransferases catalyze the chain elongation of allylic pyrophosphate (usually farnesyl pyrophosphate; FPP) *via* sequential condensation reactions with isopentenyl pyrophosphate (IPP; Liang *et al.*, 2002). The polyprenyl pyrophosphate products such as steroids, carotenoids, terpenoids, lipid carriers, natural rubber and the side chains of respiratory quinones serve a variety of important biological functions (Liang *et al.*, 2002). During each condensation reaction of IPP, a new double bond is formed. Prenyltransferases have been classified as *E*- and *Z*-types, which catalyze the formation of *trans* and *cis* double bonds, respectively. Octaprenyl pyrophosphate synthase (OPPs) is an *E*-type prenyltransferase found in bacteria that catalyzes the condensation of five IPP molecules with FPP to generate C_{40} octaprenyl pyrophosphate (OPP; Fujisaki *et al.*, 1986; Asai *et al.*, 1994), which constitutes the side chain of ubiquinone, which is involved in electron-transfer reactions (Okada *et al.*, 1996). OPPs has been demonstrated to be essential for bacterial growth owing to its significant role in the bacterial life cycle (Okada *et al.*, 1997; Apfel *et al.*, 1999).

OPPs, along with most of the *Z*-type prenyltransferases, synthesize long-chain products. In the crystal structure of undecaprenyl pyrophosphate synthase (UPPs), an elongated crevice covered with hydrophobic amino acids was proposed as the substrate/product binding site (Chang *et al.*, 2003; Ko *et al.*, 2001; Fujihashi *et al.*, 2001). We have previously demonstrated that Triton X-100 can increase the enzyme activity, accelerating release of the product (Pan *et al.*, 2000), and a polyprenyl carrier protein was also proposed to aid the product release (Ogura *et al.*, 1997).

Three-dimensional structural information about prenyltransferases is required for the understanding of the mechanism and function of these enzymes. Although the structure of FPPs, a short-chain *E*-type enzyme, has already been solved (Tarshis *et al.*, 1994), no structure of a long-chain *E*-type prenyltransferase is yet available. Therefore, we expressed and crystallized two OPPs from the mesophilic *Escherichia coli* and, in order to increase chances of enzyme crystallizability, from the hyperthermophilic *Thermatoga maritima* (the enzymes share 28% sequence identity; Fig. 1). Subsequently, we have determined the crystal structure of *T. maritima* OPPs at 2.8 Å resolution by the multiple isomorphous replacement method (MIR).

2. Materials and methods

2.1. Protein preparation

2.1.1. *T. maritima* OPPs. The *T. maritima* OPPs gene that encodes 299 amino-acid residues was amplified from genomic DNA by the polymerase chain reaction (PCR) and inserted into the vector *pET*-32*Xa/LIC* (Novagen)

under the control of the inducible T7 promotor. The recombinant plasmid was then transformed into host *E. coli* BL21 (DE-3) (Novagen) for expression. The procedure for protein purification followed a previously reported protocol (Kuo & Liang, 2002). The purified *T. maritima* OPPs was verified by mass-spectroscopic analysis and its purity (>95%) was checked by SDS–PAGE.

2.1.2. *E. coli* OPPs. The *E. coli* OPPs gene of 323 amino-acid residues was amplified by PCR and inserted into the same vector as for the *T. maritima* OPPs and the protein purified by a previously reported protocol (Pan *et al.*, 2002). However, this protein could not be crystallized after initial screening. To obtain more pure protein, we produced a new construct with the *E. coli* OPPs gene inserted into the vector pET16b. An extra 20 residues including a ten-His tag were added to the N-terminus of the *E. coli* OPPs protein. The protein was purified from an Ni-NTA column, concentrated by Amicon (Millipore) and lyophilized and stocked after salt removal using a HiPrep 26/10 desalting column (Amersham Biosciences).

2.2. Crystallization and soaking with heavy atoms

Initial crystallization screening was performed using Hampton Research Crystal Screens (Laguna Niguel, CA, USA) with the hanging-drop vapour-diffusion method. In general, 2 µl of solution containing *T. maritima* OPPs or *E. coli* OPPs solution [25 m*M* Tris–HCl, 150 m*M* NaCl pH 7.5, 0.2%(*v*/*v*) Triton X-100] was mixed with 2 µl of reservoir solution and the mixture was maintained at 300 K. Crystallization was carried out with *T. maritima* OPPs or *E. coli* OPPs concentrations between 5 and 10 mg ml^{-1}.

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          1                                                  43
Thermo    MTKNKLNQ.N SYELEKVKER I.EQILSQFF PEQIMKDLPL Y.....GKML
E.coli    MNLEKINELT AQDMAGVNAA ILEQLNSDV. ..QLINQLGY YIVSGGGKRI

          44                                                 93
Thermo    RVRLSILSFK NRGVEIGEDA ISSLAALELV HLASLLHDDV IDGARFRRGK
E.coli    RPMIAVLAAR AVGYE.GNAH VTIAALIEFI HTATLLHDDV VDESDMRRGK

          94                                                 143
Thermo    ETINFMYGDK AAVAAGDLVL VSAFHTVEEI GNNKLRRAFL NVIGKMSEAE
E.coli    ATANAAFGNA ASVLVGDFIY TRAFQMMTSL GSLKVLEVMS EAVNVIAEGE

          144                                                189
Thermo    LIEQLSRYKP .ITKEEYLRI VEGKSGALFG LALQLPALLE G...ELGEDL
E.coli    VLQLMNVNDP DITEENYMRV IYSKTARLFE AAAQCSGILA GCTPEEEKGL

          190                                                238
Thermo    YNLGVTIGTI YQMFDDIMDF .AGMEKIGKD GFLDLKNGVA SFPLVTAMEK
E.coli    QDYGRYLGTA FQLIDDLLDY NADGEQLGKN VGDDLNEGKP TLPLLHAMHH

          239                                                273
Thermo    .FPEARQMFE NRDWSG.... ....LMSFMR EKGILK.... ..ECEETLKV
E.coli    GTPEQAQMIR TAIEQGNGRH LLEPVLEAMN ACGSLEWTRQ RAEEEADKAI

          274                          299
Thermo    LVKNVIIENS WLRDFVDGIF KIKISS..
E.coli    AALQVLPDTP W.REALIGLA HIAVQRDR
```

Figure 1
Sequence alignment of the OPPs from *T. maritima* and *E. coli*. The numbers are for the *T. maritima* (Thermo) sequence. Identical amino-acid residues are shaded grey.

Molecular replacement with the use of the FPPs as a model (PDB code 1fps) did not yield a correct solution. In order to prepare heavy-atom derivatives for MIR, the *T. maritima* OPPs crystals were soaked for 2 d in cryoprotectant solution consisting of 0.1 *M* Na HEPES pH 7.5 and 2.5 *M* Li_2SO_4 that contained either methylmercuric acetate ($CH_3HgOOCCH_3$), mercury (II) cyanide [$Hg(CN)_2$] or tetrakis(acetoxymercuri)methane [$C(HgOOCH_3)_4$] at 2 m*M* concentrations.

2.3. Data collection and analysis

Preliminary X-ray diffraction experiments were carried out using an R-AXIS IV^{++} image-plate detector (Molecular Structure Corporation, The Woodlands, TX, USA) and Cu $K\alpha$ radiation generated by a Rigaku MicroMax007 rotating-anode generator. Higher resolution X-ray data were collected using synchrotron radiation and an ADSC Quantum 4 CCD camera at the BL12B2 Taiwan beamline at SPring-8, Japan. Data were processed using the *HKL* software package (Otwinowski & Minor, 1997).

Heavy atoms in the *T. maritima* OPPs crystals were located using the program *SOLVE* (Terwilliger & Berendzen, 1999), which was also used for calculation of the phase angles. The MIR map at 2.8 Å was subjected to maximum-likelihood density modification followed by autotracing using *RESOLVE* (Terwilliger, 2000). An initial model was built using *RESOLVE* and *XtalView* (McRee, 1999). The model was improved by manual rebuilding using *XtalView* and was refined using *CNS* (Brünger *et al.*, 1998).

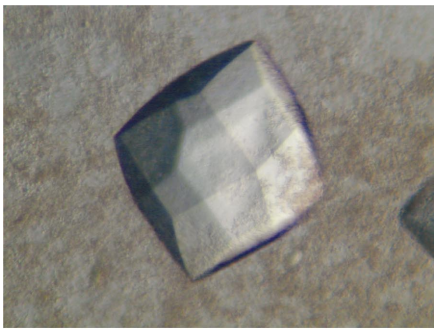
3. Results and discussion

As shown in Fig. 2(*a*), large single *T. maritima* OPPs crystals were obtained in 0.1 *M* Na HEPES pH 7.5 and 1.5 *M* Li_2SO_4. Prior to data collection at 100 K, the crystals were mounted in a cryoloop and flash-frozen in liquid nitrogen with the addition of Li_2SO_4 to 2.5 *M* as a cryoprotectant. Crystals of *T. maritima* OPPs belong to the tetragonal space group $P4_12$, with unit-cell parameters $a = b = 151.53$, $c = 69.72$ Å. Assuming two molecules per asymmetric unit, the Matthews coefficient V_M (Matthews, 1968) is 2.86 Å^3 Da^{-1}, giving a solvent content of 55%.

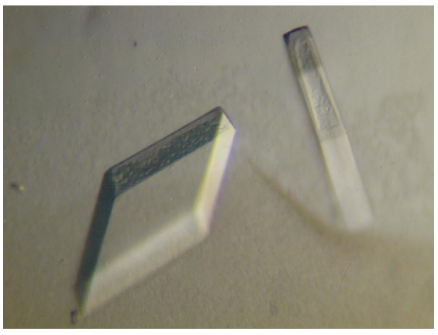
Crystals of *E. coli* OPPs (Fig. 2*b*) were obtained in 0.1 *M* citric acid pH 5.0 and 0.8 *M* $(NH_4)_2SO_4$. For data collection, these crystals were mounted and flash-frozen with the addition of 30%(*v*/*v*) glycerol as a

cryoprotectant. The *E. coli* OPPs crystals belong to the *C*-centred orthorhombic space group $C222_1$, with unit-cell parameters a = 247.66, b = 266.10, c = 157.93 Å. Assuming ten, 12 or 14 molecules per asymmetric unit would give corresponding V_M values of 3.46, 2.88 or 2.47 Å^3 Da^{-1}, respectively, and solvent contents of 63.1, 57.3 or 48.31%, respectively. Some data-collection statistics for the *T. maritima* and *E. coli* OPPs are listed in Table 1.

For MIR phasing, three data sets of heavy-atom derivatives of *T. maritima* OPPs crystals were used. Statistical values for data collection and phasing are listed in Table 2. The initial electron-density map clearly revealed that the asymmetric unit contains two molecules of OPPs in the form of a dimer and that the protein consists mostly of α-helices. After autotracing by *RESOLVE*, a model with 280 amino-acid residues including all side chains in each subunit was fitted into the electron densities (Fig. 3). The overall protein fold of *T. maritima* OPPs is similar to that of avian FPPs (Tarshis *et al.*, 1994), despite their limited sequence identity (19%). The current R and R_{free} values are 0.25 and 0.32 for all 2.28 Å resolution data, respectively, and further refinement is in progress.



(*a*)



(*b*)

Figure 2
Crystals of octaprenyl pyrophosphate synthase (OPPs) from *T. maritima* (*a*) and *E. coli* (*b*) used in data collection; the approximate dimensions of the crystals are (*a*) 0.5 × 0.5 × 0.2 mm and (*b*) 0.7 × 0.3 × 0.1 mm.

Table 1
Data-collection statistics of the native OPPs crystals from *T. maritima* and *E. coli*.

Values in parentheses are for the highest resolution shell.

Data set	OPPs from *T. maritima*	OPPs from *E. coli*
Space group	$P42_12$	$C222_1$
Unit-cell parameters (Å)	a = 151.53, b = 151.53, c = 69.72	a = 247.66, b = 266.10, c = 157.93
Resolution (Å)	50–2.28 (2.36–2.28)	50–3.9 (4.0–3.9)
No. of observations	360254 (35095)	391520 (33251)
Unique reflections	36239 (3529)	47783 (4724)
Completeness (%)	96.3 (95.8)	99.9 (99.9)
R_{merge} (%)	5.9 (42.3)	11.6 (44.6)
Average $I/\sigma(I)$	31.48 (4.36)	17.38 (3.90)

Table 2
Heavy-atom derivatives and MIR statistics of the *T. maritima* OPPs crystal.

Values in parentheses are for the highest resolution shell.

Data set	$CH_3HgOOCCH_3$	$Hg(CN)_2$	$C(HgOOCH_3)_4$
Space group	$P42_12$		
Unit-cell parameters (Å)	a = b = 151.29, c = 69.16	a = b = 150.76, c = 69.60	a = b = 151.07, c = 69.10
Resolution (Å)	50–2.7 (2.8–2.7)	50–2.8 (2.9–2.8)	50–2.8 (2.9–2.8)
No. of observations	153135 (13429)	144563 (11332)	145154 (10162)
Unique reflections	22584 (2205)	20151 (1936)	20174 (1880)
Completeness (%)	99.7 (99.6)	99.0 (97.2)	99.4 (95.0)
R_{merge} (%)	6.7 (44.3)	6.1 (24.3)	6.4 (36.3)
Average $I/\sigma(I)$	22.53 (4.25)	25.57 (5.55)	27.23 (4.39)
Phasing power†	0.76	0.53	0.58
Mean overall FOM	0.50 (50–2.8 Å)		
No. of sites	4	2	2

† Phasing power is the ratio of the r.m.s. of the heavy-atom scattering amplitude and the lack-of-closure error.

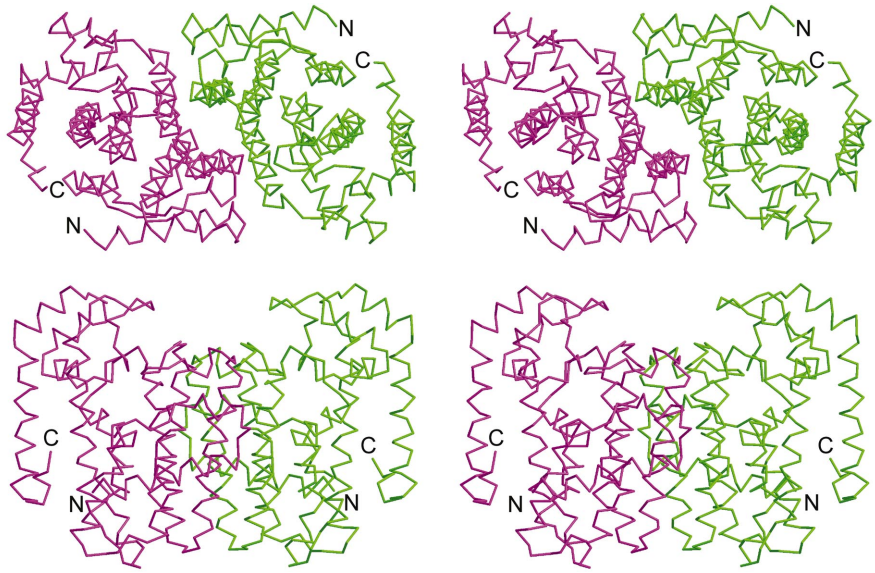


Figure 3
The α-carbon tracing of the model of the *T. maritima* OPPs dimer; different subunits are coloured pink and green and the model is shown in two orthogonal views. This figure was prepared using *MolScript* (Kraulis, 1991) and *Raster3D* (Merritt & Bacon, 1997).

Because of the large unit cell and the large number of protein molecules in the asymmetric unit and the lack of well defined non-crystallographic symmetry, structure determination of the *E. coli* OPPs crystals is not straightforward. We are working on heavy-atom derivatives and trying to solve the structure using the MIR approach. Structure determination by molecular replacement is also in progress. In addition, we are preparing fresh *E. coli* OPPs protein and searching for new crystallization conditions to obtain new crystal forms in different space groups, hopefully with fewer molecules in the unit cell.

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