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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}.

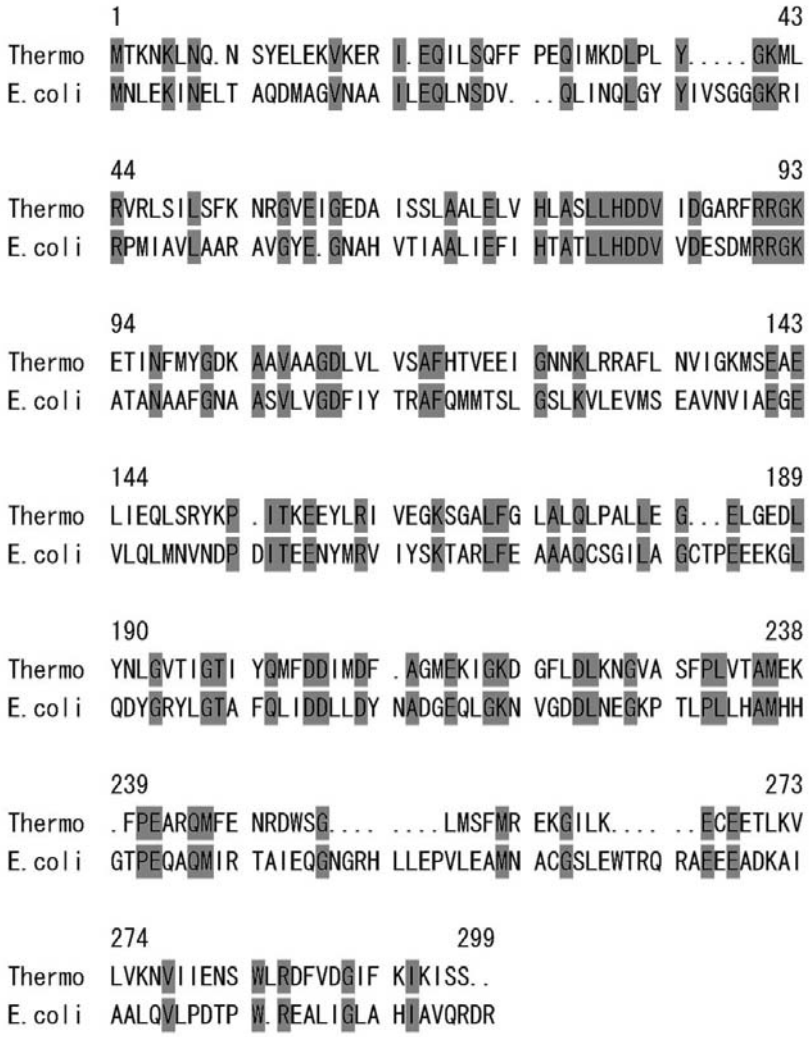

Figure 1
Sequence alignment of the OPPs from *T. maritima* and *E. coli*. The numbers are for the *T. maritima* (Thermo) sequence. Identical amino-acid residues are shaded grey.

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.

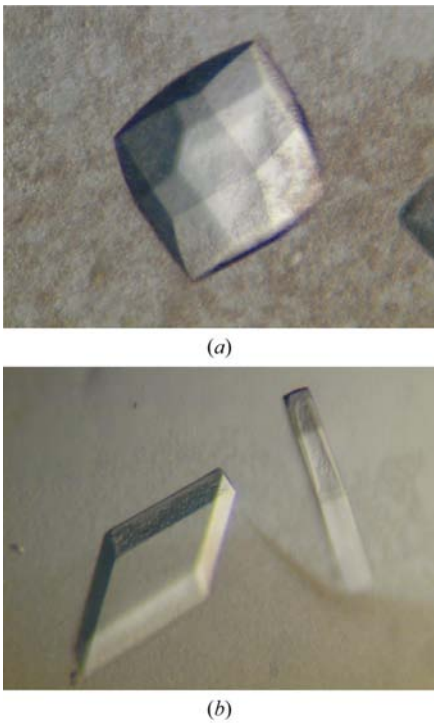

Figure 2
Crystals of octaprenyl pyrophosphate synthase (OPPs) from *T. maritima* (*a*) and *E. coli* (*b*) used in data collection; the approximate dimensions of the crystals are (*a*) 0.5 × 0.5 × 0.2 mm and (*b*) 0.7 × 0.3 × 0.1 mm.

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.

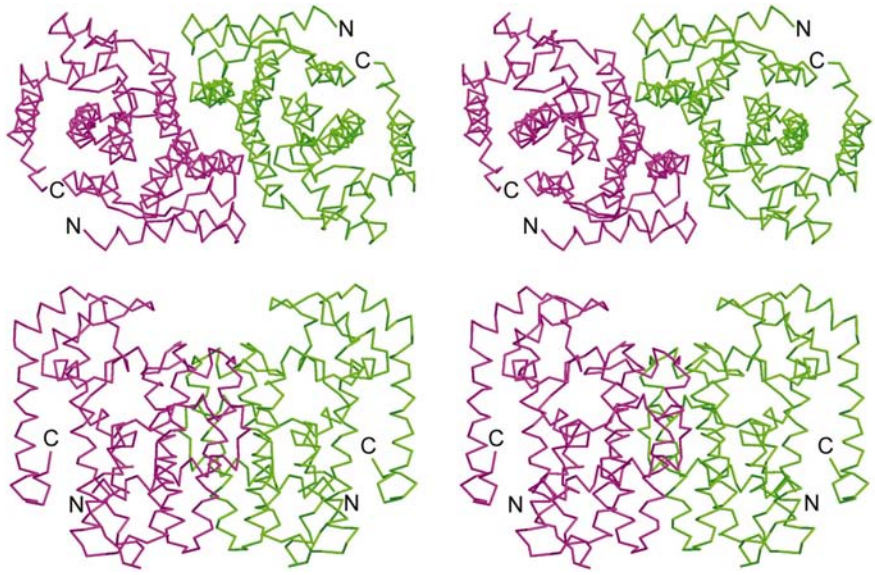

Figure 3
The α-carbon tracing of the model of the *T. maritima* OPPs dimer; different subunits are coloured pink and green and the model is shown in two orthogonal views. This figure was prepared using *MolScript* (Kraulis, 1991) and *Raster3D* (Merritt & Bacon, 1997).

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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