Preliminary X-ray diffraction analysis of octaprenyl pyrophosphate synthase from *Escherichia coli*

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Octaprenyl pyrophosphate synthase (OPPs), which belongs to the *E*-type prenyltransferase family, catalyses the successive condensation of farnesyl pyrophosphate with five isopentenyl pyrophosphate molecules to form *trans*-C₄₀-octaprenyl pyrophosphate (OPP). OPP is essential for the biosynthesis of bacterial ubiquinone or menaquinone side chains, which play an important role in the electron-transport system. Here, *Escherichia coli* OPPs was expressed, purified and crystallized. The crystals, which belonged to the orthorhombic space group *P*₂₁₂₁₂, with unit-cell parameters *a* = 117.0, *b* = 128.4, *c* = 46.4 Å, were obtained by the sitting-drop vapour-diffusion method and diffracted to 2.2 Å resolution. Initial phase determination by molecular replacement (MR) clearly indicated that the crystal contained one homodimer per asymmetric unit. Further model building and structural refinement are in progress.

1. Introduction

Prenyltransferases transfer the allylic groups from designated numbers of isopentenyl pyrophosphate (IPP) to farnesyl pyrophosphate (FPP) for chain elongation to yield polyprenyl pyrophosphate products (Kellogg & Poulter, 1997; Ogura & Koyama, 1998; Søballe & Poole, 1999; Liang *et al.*, 2002). These natural products, including carotenoids, steroids, terpenoids and the lipid carriers, play important roles in biological functions and signalling pathways (Gershenson & Dudareva, 2007; Kirby & Keasling, 2009). Prenyltransferases are classified into *E* and *Z* types: *E*-type enzymes form *trans*-double bonds and *Z*-type enzymes form *cis*-double bonds. Octaprenyl pyrophosphate synthase (OPPs), belonging to the *E*-type prenyltransferase family, catalyses the sequential condensation reaction of FPP with five IPP molecules to generate *trans*-C₄₀-octaprenyl pyrophosphate (OPP) (Liang *et al.*, 2002). Recently, a homology model of the *Escherichia coli* OPPs structure was built from the *Thermotoga maritima* OPPs structure (PDB code 1v4e, Guo *et al.*, 2004) using MODELLER (Sali & Blundell, 1993) and structure-based mutagenesis studies of *E. coli* OPPs have been conducted to investigate the substrate-binding pattern and catalysis machinery (Chang *et al.*, 2012). Although the modelling study provides some information for the underlying mechanism of *E. coli* OPPs, structure analyses of the protein and substrate-bound complex are required to fully understand the catalytic and chain determination mechanism.

We have previously obtained crystals of *E. coli* OPPs (Guo *et al.*, 2003), but they were not suitable for X-ray analysis for several reasons. First, the unit-cell dimensions were too large (*a* = 247.66, *b* = 266.10, *c* = 157.93 Å) and an asymmetric unit might contain 10, 12 or 14 protein molecules as estimated by the Matthews coefficient (Matthews, 1968). Second, the diffraction resolution was too low (about 3.9 Å) and no suitable model was available for molecular replacement (MR). The model with the highest identity of 28% was *T. maritima* OPPs at that time (Guo *et al.*, 2003). We have been searching for crystals with smaller cell dimensions and higher resolution by employing other crystallization conditions. Here we report a new crystal form of *E. coli* OPPs that diffracted X-rays to 2.2 Å. The structure was then solved by MR with the *E. coli* OPPs model generated from the SWISS-MODEL website using the structure of *Rhodobacter capsulatus* decaprenyl pyrophosphate synthase (PDB

Table 1Data-collection statistics for the *E. coli* OPPs crystal.

Values in parentheses are for the highest-resolution shell.

Beamline	BL13C1, NSRRC
Wavelength (Å)	0.97622
Resolution (Å)	25–2.2 (2.28–2.20)
Space group	$P2_12_12$
Unit-cell parameters (Å)	$a = 117.0, b = 128.4, c = 46.4$
No. of measured reflections	142825 (13763)
No. of unique reflections	36335 (3529)
Completeness (%)	99.8 (99.4)
$R_{\text{merge}}^{\dagger}$ (%)	5.8 (51.7)
Mean $I/\sigma(I)$	27.5 (3.0)
Multiplicity	3.9 (3.9)
Detector	MX300HE
I/σ criterion	5
X-ray beam size (μm)	200
Oscillation range (°)	0.5
Exposure time (s)	5
Crystal-to-detector distance (mm)	300

$$\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

code 3mvz, New York SGX Research Center for Structural Genomics, unpublished work) as a template model.

2. Materials and methods

2.1. Protein preparation

Protein expression and purification followed the previous procedures with slight modifications (use of a new vector, pET46 Ek/LIC) (Guo *et al.*, 2003). Briefly, the gene encoding OPPs (protein ID AEG38106.1) from *E. coli* was amplified by polymerase chain reaction (PCR) with a forward primer 5'-GACGACGACAA-GATGAATTTAGAAAAATCAATGAGTTAACC-3' and a reverse primer 5'-GAGGAGAAGCCCGTTAATTAACGATCG-CGTTGAACAGCGATGT-3', and then cloned into the vector pET46 Ek/LIC. The recombinant plasmid was transformed into *E. coli* BL21E. coli OPPs protein was induced with 0.8 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 310 K for 4 h. The cell pellet was harvested by centrifugation at 6000g and resuspended in lysis buffer consisting of 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 20 mM imidazole. Cell lysate was prepared with a JNBIO pressure cell (JN-3000 PLUS) and then centrifuged at 17 000g to remove cell debris. The target protein was purified on an ÄKTA-Purifier 10 (GE Healthcare Life Sciences) using a Ni-NTA column. The buffer used for the Ni-NTA column was 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 20 mM imidazole. The target protein was eluted at about 80 mM imidazole when using a 20–250 mM imidazole gradient. The protein solution was dialysed twice against 5 l buffer consisting of 25 mM Tris-HCl pH 7.5, and loaded onto a 20 ml DEAE Sepharose Fast Flow column (GE Healthcare Life Sciences). The buffer and gradient were 25 mM Tris-HCl pH 7.5 and 0–500 mM NaCl, respectively. The eluted *E. coli* OPPs (35 kDa; amino acids 1–323) was then dialysed twice against 5 l buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl) and concentrated to 3 mg ml⁻¹ using an Amicon Ultra-15 Centrifuge (Millipore); the purity was checked by SDS-PAGE analysis (>95%).

2.2. Crystallization and data collection

Subsequent crystallization screening was performed manually using 768 different reservoir conditions from Hampton Research (Laguna Niguel, CA, USA) including Crystal Screen, Crystal Screen 2, Crystal Screen Cryo, Crystal Screen Lite, MembFac, Natrix, Index,

SaltRx, SaltRx 2, PEG/Ion Screen, PEG/Ion 2 Screen, Quick Screen and Grid Screen (ammonium sulfate, MPD, sodium chloride, sodium malonate, PEG 6000, PEG/LiCl). All of the crystallization experiments were conducted at 295 K using the sitting-drop vapour-diffusion method. In general, 2 μl of *E. coli* OPPs-containing solution (25 mM Tris-HCl, 150 mM NaCl pH 7.5; 3 mg ml⁻¹) was mixed with 2 μl of reservoir solution in 24-well Cryschem plates (Hampton Research) and equilibrated against 300 μl of the reservoir solution. New crystals of *E. coli* OPPs appeared within 3 d using the Index condition No. 85 [0.2 M magnesium chloride hexahydrate, 0.1 M Tris-HCl pH 8.5, 25%(w/v) PEG 3350]. The crystallization condition was optimized by using 0.3 M magnesium chloride hexahydrate, 0.1 M Tris-HCl pH 8.5, 24%(w/v) PEG 3350. Within 3–4 d, the crystals reached dimensions of about 0.1 × 0.1 × 1.5 mm. Prior to data collection at 100 K, the crystal was mounted in a cryoloop and soaked with cryoprotectant solution which consisted of 0.3 M magnesium chloride hexahydrate, 0.1 M Tris-HCl pH 8.5, 28%(w/v) PEG 3350 and 4%(v/v) glycerol for 3 s. An X-ray diffraction data set was collected to 2.2 Å resolution on beamline BL13C1 of the National Synchrotron Radiation Research Center (NSRRC, Hsinchu, Taiwan). The diffraction images were processed using the program HKL-2000 (Otwinowski & Minor, 1997). Data-collection statistics are given in Table 1.

3. Results and discussion

Previously, we had made some different constructs of *E. coli* OPPs (with a ten-His tag and ten cloning-artifact residues by using pET16; or without the 20-residue tag by using pET32 Xa/LIC and removing the tag by Xa digestion). We screened for as many conditions as possible at different temperatures (300, 298, 289, 277 K); the protein buffer was also tested with and without 0.2%(v/v) Triton X-100 in 25 mM Tris-HCl pH 7.5, 150 mM NaCl. Several different crystals were obtained from some NaCl and PEG conditions, but all of these crystals belonged to the same space group ($C222_1$) (Guo *et al.*, 2003). With 10–14 molecules in the asymmetric unit, and a resolution of 3.9 Å, it was not possible to solve the crystal structure. In the end, we solved the crystal structure of *T. maritima* OPPs instead (PDB code 1v4e, Guo *et al.*, 2004).

Figure 1

A crystal of *E. coli* OPPs. The crystal reached approximate dimensions of 0.1 × 0.1 × 1.5 mm in 3–4 d.

Figure 2
A diffraction pattern of the *E. coli* OPPs crystal.

Several years later, as a starting project after moving from Taipei, Taiwan to Tianjin, China and setting up a new laboratory, we tried to see if *E. coli* OPPs could be crystallized in other space groups with a different packing. The construct we used here is only a little different from the previous one, but it might not be the critical point. We could obtain the same form of crystals ($C222_1$) whether the protein construct had an extra 20 residues at the N-terminus or not. In fact, this time, we used a new construct (pET46 EK/LIC) which contains an extra 14 amino-acid residues (MAHHHHHHVDDDDK) at the N-terminus. However, the crystal structure model of *E. coli* OPPs starts at the first amino acid and no density for extra residues can be seen. In addition, there is no interaction between the N-terminal residues of *E. coli* OPPs and any symmetry-related molecule. Thus

the extra N-terminal residues are probably not involved in crystal packing.

Many factors (temperature, humidity *etc.*) influence crystal growth in ways that are still poorly understood. We were successful this time in obtaining well diffracting crystals of *E. coli* OPPs which may be due to the different crystal-growing environment. The humidity in Taipei is about 80% whereas it is 20% in Tianjin. Some other subtle changes may also have influenced the crystallization. We are not sure why we could grow a new crystal form but the change in location is a possible explanation.

One of the rod-shaped single OPPs crystals is shown in Fig. 1. On the basis of the diffraction pattern (Fig. 2), these *E. coli* OPPs crystals belong to the orthorhombic space group $P2_12_12$ with unit-cell parameters $a = 117.0$, $b = 128.4$, $c = 46.4$ Å. Assuming there are two molecules per asymmetric unit, the Matthews coefficient V_M is $2.50 \text{ Å}^3 \text{ Da}^{-1}$ and the estimated solvent content is 50.7% (Matthews, 1968).

The crystal structure of *E. coli* OPPs was solved by the MR method with the *Phaser* program (McCoy *et al.*, 2007) from the *CCP4* suite (Winn *et al.*, 2011) using a hypothetical *E. coli* OPPs model as the search model. This model was generated from the structure of *Rhodobacter capsulatus* decaprenyl pyrophosphate synthase (PDB code 3mzv; 45.2% sequence identity with *E. coli* OPPs) by the SWISS-MODEL website. Preliminary structural refinement using *REFMAC5* (Murshudov *et al.*, 1997) and *CNS* (Brünger *et al.*, 1998) resulted in a model with R_{work} and R_{free} of 33 and 38%, respectively. There are two monomers in the asymmetric unit, forming an active homodimer, which is consistent with a previous study (Kainou *et al.*, 2001) and like other known *E*-type prenyltransferases (Oldfield & Lin, 2012). A crystal packing diagram is shown in Fig. 3. Further model building and structural refinement are underway. Finally, in an attempt to fully understand the catalytic and chain-length determination, co-crystallizing and/or soaking the *E. coli* OPPs crystals with its substrates IPP, FPP or its substrate analogue farnesyl thio-pyrophosphate (FsPP) are underway.

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Figure 3
Unit-cell contents of the *E. coli* OPPs crystal. In this stereoview, four homodimers are shown as C_α tracing models in blue, red, green and grey. The unit cell is shown as a box with a horizontal a axis and vertical b axis.

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