Expression, crystallization and preliminary
crystallographic study of octaprenyl pyrophosphate
synthase from *Helicobacter pylori*Jinyong Zhang,^a Xiaoli Zhang,^a
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Octaprenyl pyrophosphate synthase (OPPs) is involved in the synthesis of the side chains of ubiquinone and menaquinone and catalyzes consecutive condensation reactions of farnesyl pyrophosphate with isopentenyl pyrophosphate to generate polyprenyl pyrophosphate and pyrophosphate. In order to investigate the roles played by OPPs in the metabolism of ubiquinone and menaquinone and the enzymatic mechanisms of these enzymes, analysis of the structure–function relationship of OPPs from *Helicobacter pylori* was initiated. The gene for OPPs was cloned, the protein was expressed, purified and crystallized and a diffraction data set was collected to 2.00 Å resolution. The crystals belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 109.33$, $c = 103.41$ Å.

1. Introduction

Coenzyme Q₁₀ (CoQ₁₀), also known as ubiquinone, is one of the most important nutrients for most life forms (Ely & Krone, 2000) and is a component of the electron-transport chain (Sohal *et al.*, 2006). It contains a quinone chemical group and a condensative polyprenyl tail. In its name, the character 'Q' refers to the quinone chemical group and the number '10' refers to the number of isoprenyl chemical subunits, which varies from six to ten. CoQ₁₀ is found in plants and mammals and coenzymes Q₆, Q₇ and Q₈ are found in yeast and bacteria, whereas CoQ₉ is found in rats and mice. The isoprenyl tail of bacterial ubiquinone or menaquinone is synthesized by octaprenyl pyrophosphate synthase (OPPs) and homologous enzymes such as hexaprenyl pyrophosphate synthase (HexPPs), heptaprenyl pyrophosphate synthase (HepPPs), solanesyl diphosphate synthase (SPPs) and decaprenyl diphosphate synthase (DPPs). The synthesis of side chains with different numbers of isoprenyl chemical subunits (six, seven, eight, nine and ten) are proposed to be performed by HexPPs, HepPPs, OPPs, SPPs and DPPs, respectively. For example, OPPs as reported here catalyzes the synthesis of *trans*-C₄₀-octaprenyl pyrophosphate (OPP) by the reaction of five isopentenyl pyrophosphate (IPP) molecules with one farnesyl pyrophosphate (FPP) and the product could be incorporated in the side chain of Q₈.

OPPs and homologous proteins belong to a class of enzymes named prenyltransferases, which are a wide array of enzymes that are involved in the synthesis of linear isoprenyl pyrophosphates and that catalyze the condensation reaction of IPP with allylic substrates such as the C₅ dimethylallyl pyrophosphate (DMAPP), the C₁₅ FPP or the C₂₀ geranylgeranyl pyrophosphate (GGPP) for chain elongation using the five-carbon IPP as a building block (Søballe & Poole, 1999; Liang *et al.*, 2002). The products of varying chain length, such as steroids, carotenoids, quinones, dolichols, prenylated proteins and archaeal membrane lipids, are then utilized as precursors for many compounds involved in a variety of essential biological functions and signalling pathways (Sun *et al.*, 2005). According to the stereochemical outcome of their products, prenyltransferases can be classified as *cis*- and *trans*-prenyltransferases (Kharel & Koyama, 2003; Ogura & Koyama, 1998). The *cis*-prenyltransferases are involved in catalyzing the synthesis of larger products from C₅₅ to C₁₂₀ from farnesyl pyrophosphate, while *trans*-prenyltransferases generally generate products shorter than C₅₅ from farnesyl

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Table 1

X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 109.33$, $c = 103.41$
Wavelength (Å)	0.97901
Resolution (Å)	54.63–2.00 (2.11–2.00)
No. of reflections	42833
$R_{\text{merge}}^{\dagger}$	9.0 (36.8)
Multiplicity	26.3 (15.7)
$\langle I/\sigma(I) \rangle$	31.5 (6.8)
Completeness (%)	99.9 (99.3)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $\langle I(hkl) \rangle$ is the mean of the observations $I_i(hkl)$ of reflection hkl .

pyrophosphate (Asawatreratanakul *et al.*, 2003; Sato *et al.*, 1999). OPPs belongs to the latter.

To date, there have been no reports on the relationship between the synthesis of the CoQ side chain and the structure of the corresponding *trans*-prenyltransferase, even though more than ten crystal structures from the *trans*-prenyltransferase family have been solved. Interestingly, there was found to be only one OPPs and no HexPPs, HepPPs, SPPs or DPPs in *Helicobacter pylori* from scanning the genome. In order to better understand the role of *trans*-prenyltransferases in the synthesis of CoQ and the enzymatic mechanism, we have therefore embarked on the structure determination of OPPs from *H. pylori*. Here, we report the crystallization, diffraction data collection and preliminary crystallographic studies of OPPs from *H. pylori*.

2. Materials and methods

2.1. Cloning, expression and purification of *H. pylori* OPPs

The coding sequence of the OPPs gene (NCBI Gene ID 899975) was amplified by PCR from *H. pylori* strain 11637 genomic DNA and cloned into the pET22b vector (Novagen) *via* introduced *NdeI/XhoI* restriction sites, resulting in the recombinant plasmid pET22b(+): OPPs containing a sequence coding for a hexahistidine tag (LEH-HHHHH) fused to the 3'-end of the OPPs gene. *Escherichia coli* strain BL21 (DE3) competent cells were transformed with the recombinant plasmid and grown in 1.0 l LB medium at 310 K with

Figure 1

H. pylori OPPs crystals grown by the hanging-drop method in 15% polyethylene glycol 3350, 100 mM sodium acetate pH 4.8 and 200 mM MgCl_2 . Typical crystal dimensions were $0.1 \times 0.1 \times 0.5$ mm.

$100 \mu\text{g ml}^{-1}$ ampicillin. When the OD_{600} of the culture reached 0.8, the cells were induced with 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) at 298 K overnight and were then harvested by centrifugation at $4000 \text{ rev min}^{-1}$ for 40 min at 277 K. The cell pellets were resuspended in 15 ml lysis buffer (50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl, 10 mM imidazole) and sonicated on ice. The lysate was centrifuged at $16\,000 \text{ rev min}^{-1}$ for 25 min at 277 K. The supernatant was loaded onto a Ni-NTA column (Novagen) equilibrated with lysis buffer. After washing with washing buffer (50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl, 25 mM imidazole) to remove unbound fractions, elution buffer (50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl, 250 mM imidazole) was applied. The eluate containing the recombinant OPPs was concentrated to about 1.0 ml and applied onto a HiLoad 16/60 Superdex 200 prep-grade gel-filtration column (Amersham Biosciences) equilibrated with 20 mM Tris-HCl pH 7.5, 150 mM NaCl. The peak fractions from size exclusion corresponding to the recombinant protein were pooled and concentrated using an Amicon Ultra-15 Centrifugal Filter Unit (Millipore) prior to crystallization. The purified OPPs was stored at 277 K for further crystallization experiments.

2.2. Crystallization

The concentration of *H. pylori* OPPs used in crystallization was approximately 10 mg ml^{-1} . Initial crystallization screening was performed with Index, Crystal Screen and Crystal Screen 2 from Hampton Research at 293 K using the hanging-drop vapour-diffusion technique by mixing 1 μl protein solution and 1 μl reservoir solution and equilibrating against 0.5 ml reservoir solution. The crystallization condition was optimized by varying the type and the concentration of precipitant, salts and buffers and the pH.

2.3. Data collection and processing

Diffraction data were collected on beamline 17A at the Photon Factory (Tsukuba, Japan) using an ADSC Quantum 270 CCD detector. Prior to data collection, crystals were transferred into a

Figure 2

A typical diffraction pattern of an *H. pylori* OPPs crystal.

cryoprotectant solution consisting of 15% polyethylene glycol 3350, 0.2 M MgCl₂, 10% (v/v) glycerol and 0.1 M sodium acetate pH 4.8 and soaked for about 15 s. Crystals were then mounted in cryoloops (Hampton Research) for X-ray data collection and flash-cooled in a nitrogen-gas stream at 95 K. A native data set was collected to 2.00 Å resolution with a total of 360 images using an oscillation of 1° per image and a wavelength of 0.97901 Å. *MOSFLM* (v.7.0.4; Leslie, 2006) and *SCALA* (v.6.0) from the *CCP4* program suite (v.6.0.2; Collaborative Computational Project, Number 4, 1994) were used for the indexing, integration and scaling of the diffraction data.

3. Results

In the initial screening, microcrystals were obtained using Hampton Research Index condition No. 40 (25% PEG 3350, 0.1 M citric acid pH 3.5) and Crystal Screen condition No. 20 (0.2 M ammonium sulfate, 0.1 M sodium acetate pH 4.6 and 25% PEG 4000) at 293 K. Optimization of the conditions yielded well shaped crystals from a solution consisting of 15% polyethylene glycol 3350, 0.1 M sodium acetate pH 4.8 and 0.2 M MgCl₂ at 277 K. Crystals appeared in the drops within 72 h with dimensions of about 0.5 × 0.1 × 0.1 mm (Fig. 1).

The data-collection statistics for the OPPs crystals are given in Table 1. The native crystal diffracted to a resolution of 2.0 Å (Fig. 2) and belonged to space group *P*4₁2₁2 or *P*4₃2₁2, with unit-cell parameters *a* = *b* = 109.33, *c* = 103.41 Å. The mature *H. pylori* OPPs consists of a single peptide chain of 307 amino-acid residues; each asymmetric unit of the cell contains one OPPs dimer corresponding

to 65 kDa, with a solvent content of 44.59% and a Matthews coefficient of 2.10 Å³ Da⁻¹.

The high quality of the data that we have collected has laid a solid foundation for structure determination of *H. pylori* OPPs. Attempts to solve the structure are now in progress.

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