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Modeling studies with *Helicobacter pylori* octaprenyl pyrophosphate synthase reveal the enzymatic mechanism of *trans*-prenyltransferases

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ABSTRACT

Octaprenyl pyrophosphate synthase (OPPs), an enzyme belonging to the *trans*-prenyltransferases family, is involved in the synthesis of C40 octaprenyl pyrophosphate (OPP) by reacting farnesyl pyrophosphate (FPP) with five isopentenyl pyrophosphates (IPP). It has been reported that OPPs is essential for bacteria's normal growth and is a potential target for novel antibacterial drug design. Here we report the crystal structure of OPPs from *Helicobacter pylori*, determined by MAD method at 2.8 Å resolution and refined to 2.0 Å resolution. The substrate IPP was docked into *Hp*OPPs structure and residues involved in IPP recognition were identified. The other substrate FPP, the intermediate GGPP and a nitrogen-containing bisphosphonate drug were also modeled into the structure. The resulting model shed some lights on the enzymatic mechanism, including (1) residues Arg87, Lys36 and Arg39 are essential for IPP binding; (2) residues Lys162, Lys224 and Gln197 are involved in FPP binding; (3) the second DDXXD motif may involve in FPP binding by Mg^{2+} mediated interactions; (4) Leu127 is probably involved in product chain length determination in *Hp*OPPs and (5) the intermediate products such as GGPP need a rearrange to occupy the binding site of FPP and then IPP is reloaded. Our results also indicate that the nitrogen-containing bisphosphonate drugs are potential inhibitors of FPPs and other *trans*-prenyltransferases aiming at blocking the binding of FPP.

1. Introduction

Octaprenyl pyrophosphate synthase (OPPs) catalyzes consecutive condensation reactions of farnesyl pyrophosphate (FPP) with five molecules of isopentenyl pyrophosphate (IPP) to yield C40 ocataprenyl pyrophosphate (OPP) (Liang et al., 2002). The enzyme belongs to a big family termed *trans*-prenyltransferases involved in synthesis of linear isoprenyl pyrophosphates (Liang et al., 2002; Soballe and Poole, 1999), which are utilized as precursors for many compounds involved in a variety of essential biological functions and signal pathways (Gershenzon and Dudareva, 2007; Kirby and Keasling, 2009). Among these, the polymer OPP servers as the polyisopentanyl side chain of bacterial ubiquinone (UQ, also known as Coenzyme Q) or menaquinone, a component involved in electron transfer for oxidative phosphorylation (Soballe and Poole, 1999). Some similar polymers, such as hexaprenyl pyrophosphate (HexPP), solanesyl pyrophosphate (SPP) and decaprenyl pyrophosphate (DPP) could also serve as the side chain of UQ and perform the same function. Referring to the number of isoprenyl chemical subunits in the side chain, ubiquinone could be divided into Q6, Q7, Q8, Q9, Q10 and so on.

Studies on the enzymatic mechanism of *trans*-prenyltransferases mainly focuses on some issues, such as the binding of substrates, the condensation reaction with a new double bond formed, the regulation of product chain length, and the kinetic profiles of the catalyzed reaction (Gabelli et al., 2006; Liang et al., 2002). Among these, the regulation of product chain length is well studied (Tarshis et al., 1996; Chang et al., 2006; Kainou et al., 2001; Guo et al., 2004). Some residues with bulky side chain at the bottom of the active site serve as the "floor" to prevent the further extension of the product chain. The residues, such as Tyr and Trp in FPPs and GGPPs, located in the fifth position before the first DDXXD motif were thought to be essential for product chain length determination. Meanwhile, the binding modes of both substrates are also well studied in OPPs (Chang et al., 2012) and the homologue FPPs and GGPPs (Artz et al., 2011; C et al., 2008; Hosfield et al., 2004), the key residues involved in substrates binding were identified. The kinetic parameters such

Abbreviations: OPPs, octaprenyl pyrophosphate synthase; OPP, octaprenyl pyrophosphate; FPP, farnesyl pyrophosphate; IPP, isopentenyl pyrophosphates.

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as k_{cat} value for the catalytic reaction, K_m value for IPP and FPP of *Escherichia coli* OPPs have been determined previously, the rate constant of each IPP condensation suggest that product release is rate limiting. Some detergents such as triton could accelerates the rate of product release but not alter the rate of IPP condensation (Pan et al., 2002). The residues or elements involved in condensation reaction are also described in *E. coli* OPPs recently, residues Arg93, Lys225, Lys225, Lys235 and Gln208 are essential for the enzymatic reaction as confirmed by structure guided site-directed mutagenesis (Chang et al., 2012).

It has been reported that OPPs is essential for bacteria's normal growth (Okada et al., 1997). Thus, it would be a potential target for treating infectious diseases caused by bacterium such as *Helicobacter pylori (H. pylori)*. Research also revealed that the function of OPPs can be substituted by its homologous from other organisms that produce distinct forms of ubiquinone, such as Q7 and Q9 (Okada et al., 1997). These results indicate that potent drugs should inhibit the binding of substrate or the condensation reaction, but not the regulation of product chain length. It is reported that nitrogen-containing bisphosphonate drugs, which were widely used to treat osteoclast-mediated bone resorption (Green, 2003) and tumor-induced hypercalcemia (Riccardi et al., 2003), may serve as potential inhibitors of farnesyl pyrophosphate synthase (FPPs) (Hosfield et al., 2004), a homologue of OPPs involved in the synthesis of FPP. Further more, Guo et al. (2007) revealed that bisphosphonate drugs exhibit a remarkably broad spectrum of binding modes as seen in FPPs inhibition, indicating that nitrogen-containing bisphosphonate drugs are also potent inhibitors of OPPs and other *trans*-prenyltransferases. To identify how OPPs catalyzes the condensation reaction and whether nitrogen-containing bisphosphonate drugs are potent inhibitors of OPPs, we therefore have embarked on structural study of OPPs from *H. pylori*, a gastric pathogen that infects more than 50% of the world population (Kusters et al., 2006).

2. Materials and methods

2.1. Protein expression and purification

The expression and purification of native *Hp*OPPs has been reported previously (Zhang et al., 2011). The Selenomethionine (Se-Met) substituted derivative of *Hp*OPPs was obtained by expression of *Hp*OPPs in the Met metabolism defective *E. coli* strain B834 (DE3). In brief, *E. coli* strain B834 (DE3) competent cells transformed with the recombinant plasmid pET22b-*Hp*OPPs were first grown in LB medium containing 100 mg/L ampicillin at 310 K until the OD_{600} of the culture reached 0.8. The cells were harvested by low-speed centrifugation, resuspended in minimal medium containing 100 mg/L ampicillin and incubated for 2 h at 310 K. Se-Met was added to the culture with a final concentration of 50 mg/L. After incubate for 30 min, the cells were induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 12 h at 298 K and harvested by centrifugation. 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 and the supernatant was applied to 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, 20 mM imidazole) to remove unbound fractions, the Se-Met substituted derivative of *Hp*OPPs was eluted with elution buffer (50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl, 250 mM imidazole). The recombinant protein was concentrated to about 1.0 ml and then applied to a Hiload16/60 Superdex200 prep-grade gel filtration column (Amersham Biosciences) equilibrated with 20 mM Tris–HCl pH7.5, 150 mM NaCl and 5 mM DTT. The fractions corresponding to the protein were collected and assayed by SDS-PAGE. The fractions containing pure recombinant protein were pooled and concentrated using Amicon Ultra-15 Centrifugal Filter Unit (Millipore). The concentration of Se-Met substituted derivative of *Hp*OPPs was adjusted to 10 mg/ml for crystallization experiments.

2.2. Crystallization and data collection

Se-Met substituted derivative of *Hp*OPPs crystals were obtained using the hanging-drop vapor-diffusion method by mixing 1 μl of protein and 1 μl reservoir solution consisting of 16% polyethylene glycol 3350, 100 mM sodium acetate at pH 4.8, and 200 mM $MgCl_2$. Crystals appeared in the drops within 3 days with dimensions of about 0.5 mm × 0.1 mm × 0.1 mm. A single Se-Met derivatized crystal of *Hp*OPPs was used to collect MAD data sets at 2.8 Å resolution at 0.97850 Å (peak), 0.97938 Å (inflection) and 0.9700 Å (high-energy remote point). A native dataset diffract to 2.0 Å has been collected previously (Zhang et al., 2011). All these data sets were collected at Beam line 17A of Photon Factory, KEK, Japan. MOSFLM (v.7.0.4) (Leslie, 2006) and SCALA (v.6.0) from the CCP4 program suite (v.6.0.2) (1994) were used for the indexing, integration and scaling of the diffraction data sets, data statistics are summarized in Table 1.

2.3. Structure determination and refinement

The structure of *Hp*OPPs was determined by MAD method using the 2.8 Å resolution data sets. Twelve selenium atoms with occupancies above 0.5 were found and located by SHELXD (Schneider and Sheldrick, 2002). The program SOLVE (Terwilliger, 2003, 2004) was then used to refine the positions of these selenium atoms and perform initial phase calculations. After phasing, CAD and DM (Cowtan and Zhang, 1999) from the CCP4 program suite were used to merge the phase information into the native data set and perform density modification. The program Resolve (Terwilliger, 2003, 2004) was then used for solvent flattening. The initial model was constructed by using program ARP/Warp (Morris et al., 2003) with 85% of the sequence of *Hp*OPPs docked into the electron density map. The remaining residues were manually built with COOT (Emsley and Cowtan, 2004) and O (Jones et al., 1991). The model was refined using the Crystallography & NMR System (CNS) version 1.21 (Brunger et al., 1998). Throughout the refinement, five percent of the reflections were randomly chosen for R_{free} calculations and were excluded from the refinement. The model quality was checked by PROCHECK (Laskowski et al., 1996). The refinement statistics are summarized in Table 1. Figs. 1, 2, 4 and Supplementary Fig. 1 were prepared using the program PyMol (Lill and Danielson, 2011).

2.4. Molecular docking study with IPP

The AutoDock Program (version 4.2) was used for ligand-protein rigid docking calculations (Tiwari et al., 2009; Morris et al., 2008). The coordinates and topology file of IPP was obtained from the website http://xray.bmc.uu.se/hicup/. In the docking simulation, the AutoDock Tools (ADT) 4.2 was used to add the polar hydrogen, remove water molecules and ions and assign partial atomic charges with kollman charges for protein and ligand. A 60 × 80 × 50 point grid box around the central cavity of the enzyme was chosen as protein model for IPP docking. Docking for 100 number of GA runs were carried out using Lamarckian Genetic Algorithm (LGA) with all other parameters set to default. The top ranked model in the lowest energy cluster with maximum cluster size was selected for analyses.