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Crystallization and preliminary X-ray diffraction studies of undecaprenyl diphosphate synthase from *Micrococcus luteus* B-P 26

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Undecaprenyl diphosphate synthase from *Micrococcus luteus* B-P 26, one of the *Z*-prenyl chain-elongating enzymes, was crystallized using the sitting-drop vapour-diffusion method with ammonium sulfate and lithium sulfate as precipitants. The crystals belong to the monoclinic space group *C*2, with unit-cell parameters a = 127.2, b = 60.2, c = 75.7 Å, β = 105.6°. The crystals diffract X-rays to at least 2.2 Å resolution using synchrotron radiation and are suitable for high-resolution crystal structure analysis.

1. Introduction

Isoprenoid compounds are the most structurally diverse family of natural products. They serve as hormones, vitamins, pigments, electron carriers, constituents of membranes, photoreceptors and in many other roles. All isoprenoid compounds are derived from linear prenyl diphosphates, which are synthesized by sequential condensations of isopentenyl diphosphate (IPP) with allylic prenyl diphosphate (Ogura & Koyama, 1998). These condensation reactions are catalyzed by a family of prenyltransferases. Undecaprenyl diphosphate synthase (UPS), which belongs to this family, catalyzes the chain elongation using farnesyl diphosphate (FPP) as a primer to yield undecaprenyl diphosphate (UPP). This enzyme is essential for cell-wall biosynthesis in many bacteria. UPS acts as a homodimer; the subunit molecular weight of UPS from *Micrococcus luteus* B-P 26 is 29 kDa (Shimizu *et al.*, 1998).

The prenyltransferases can be classified into two major groups (*E* and *Z* types) according to the geometrical isomerism of the products. Many enzymes for the *E*-type prenyl chain elongation have been cloned and characterized (Ogura & Koyama, 1998). Multiple alignment of the amino-acid sequences of these enzymes, including farnesyl diphosphate synthase (FPS), geranylgeranyl diphosphate synthase, hexaprenyl diphosphate synthase, heptaprenyl diphosphate synthase, octaprenyl diphosphate synthase and decaprenyl diphosphate synthase, has shown the presence of seven conserved regions in the primary structures (Koyama *et al.*, 1993; Ogura & Koyama, 1998). Two of these, the second and the sixth conserved regions from the N-terminal (regions II and VI, respectively), have characteristic aspartate-rich DD*XX*D motifs. The crystal structure of avian FPS showed that the enzyme has a large central cavity formed by a bundle of ten α-helices and that the two conserved DD*XX*D motifs are located on the wall of this cavity (Tarshis *et al.*, 1994). Crystal structures of mutant FPSs in the apo state and with allylic substrates showed that the conserved DD*XX*D motif in region II plays a dominant role in the binding of these substrates through Mg^{2+} and that the chain length of the product was regulated by the depth of the central cavity of the enzyme (Ohnuma, Nakazawa *et al.*, 1996; Ohnuma, Narita *et al.*, 1996; Tarshis *et al.*, 1996).

On the other hand, the conserved regions of *Z*-type prenyl chain-elongation enzymes (*Z*-prenyltransferases) are very different from those of *E*-prenyltransferases (Shimizu *et al.*, 1998; Apfel *et al.*, 1999). UPSs from *M. luteus* B-P 26, *Escherichia coli*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, which belong to the *Z*-prenyltransferases, have five conserved regions. However, the DD*XX*D motif found in UPS from *M. luteus* B-P 26 is not conserved among the other UPSs (Shimizu *et al.*, 1998). Crystal structures of three prenyltransferase-related enzymes, 5-epi-aristolochene synthase (Starks *et al.*, 1997), squalene cyclase (Wendt *et al.*, 1997) and pentalenene synthase (Lesburg *et al.*, 1997) have shown that the substrate molecules were recognized by the shape and charge of the enzyme cleft. Although these enzymes, which use allylic diphosphates as substrates, have little overall similarity to *E*-prenyltransferases, they also use aspartate-rich motifs in binding substrates (Sacchettini & Poulter, 1997). This suggests that *Z*-prenyltransferases have a different mechanism for recognizing allylic diphosphates. The stereochemical reactions caused by *Z*- and *E*-prenyltransferases are similar to each other; a new C—C bond is formed and the C—H bond at the 2-position of IPP is cleaved on the same side as the double

bond of IPP (Ito *et al.*, 1987; Kobayashi *et al.*, 1985). The recognition mechanism for the IPP molecule bound to the active site of the *Z*-prenyltransferase is thought to be different from that of *E*-prenyltransferase.

The three-dimensional structure of UPS is, therefore, very important in understanding the molecular mechanism of this enzyme. Here, we report the crystallization and preliminary X-ray diffraction studies of UPS from *M. luteus* B-P 26.

2. Experimental, results and discussion

2.1. Purification and crystallization

UPS from *M. luteus* B-P 26 was overproduced and purified by the method previously described (Shimizu *et al.*, 1998). The cells were disrupted by sonication and the supernatant obtained after ultracentrifugation was fractionated by 30–60% saturated ammonium sulfate. The protein was further purified using an ÄKTA Explorer 10S system with MonoQ and Superdex 200 HR (Amersham Pharmacia Biotech). All purification steps were carried out at 293 K. The purity of the UPS sample was more than 95% judging from SDS–PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis).

Crystallization of UPS was carried out by the sitting-drop vapour-diffusion method at 293 K. The protein solutions contained 15 mg ml^{-1} UPS, 20 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl and 1 m*M* DTT, whereas the reservoir solutions contained 0.1 *M* sodium citrate pH 5.6, 0.5 *M* ammonium sulfate and 1.0 *M* lithium sulfate. A mixture of protein solution and an equal volume of reservoir solution were equilibrated against reservoir solution. An assembly of crystals grew in about 6 weeks to an approximate size of 1.2 × 0.8 × 0.2 mm (Fig. 1).

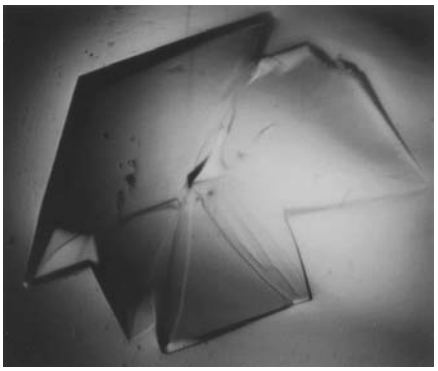

Figure 1
Crystals of undecaprenyl diphosphate synthase (UPS) from *M. luteus* B-P 26.

2.2. Data collection and processing

Single crystals with maximum dimensions 1.1 × 0.5 × 0.2 mm were cut out from the assembly and sealed in thin-walled glass capillaries with a drop of mother liquor. Preliminary diffraction data collection was performed at room temperature using synchrotron radiation at the BL6B beamline of the Photon Factory, KEK, Japan. The X-ray beam was monochromated to 1.00 Å with an Si(111) monochromator and a 0.2 mm aperture collimator was used. Oscillation photographs were taken on 400 × 800 mm imaging plates (Fuji Photo Film) on a multifunction camera for macromolecular crystallography equipped with a cylindrical cassette of 573 mm radius (Sakabe *et al.*, 1995). The image data frames were read out on a drum-type imaging-plate reader, IPR4080 (Sakabe *et al.*, 1996). The crystals diffract X-rays beyond 2.2 Å resolution (Fig. 2). A total of 18 serial oscillation images were taken, each having a rotation range of 6.3° (0.3° overlap); the exposure time for each frame was 50.4 s.

The imaging data were indexed and processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystals belong to the monoclinic space group *C*2, with unit-cell parameters $a = 127.2$, $b = 60.2$, $c = 75.7$ Å, $\beta = 105.6°$. Assuming one dimer (2 × 29 kDa) per asymmetric unit, the Matthews content, V_m, is calculated to be 2.4 Å^3 Da^{-1} and the solvent content of the crystal is calculated to be 48.8%; these are within the range commonly observed for protein crystals (Matthews, 1968).

Intensity data [$I > \sigma(I)$], consisting of 23530 unique reflections which cover 83.7% of the theoretical observations, were obtained with an $R_{\rm merge}$ of 3.2% at 2.2 Å resolution. In the highest resolution shell (2.24–2.20 Å), the $R_{\rm merge}$ and completeness were 16.8 and 56.8%, respectively. Structure analysis by means of the multiple isomorphous replacement is now in progress.

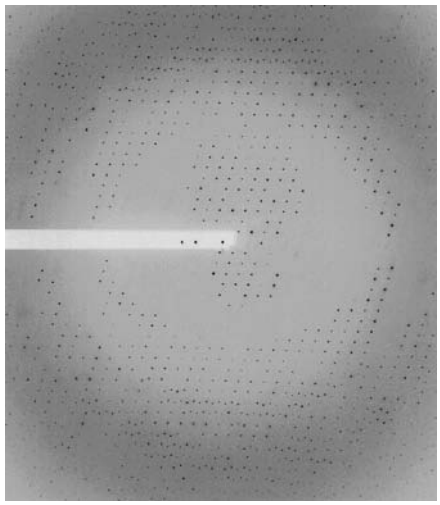

Figure 2
X-ray diffraction patterns from a crystal of UPS obtained using synchrotron radiation. The oscillation range is 6.3° and the crystal-to-film distance is 573 mm.

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