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Preliminary structural studies of *Escherichia coli* isopentenyl diphosphate isomerase

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Escherichia coli isopentenyl diphosphate isomerase, an enzyme catalyzing a key step in isoprenoid biosynthesis, has been produced in selenomethionyl form. The protein was purified and crystallized by the hanging-drop vapour-diffusion method. Crystals display trigonal symmetry, with unit-cell parameters $a = b = 71.3$, $c = 61.7$ Å, and diffract to 1.45 Å resolution.

1. Introduction

Isoprenoids are an extraordinarily diverse family of compounds in which over 23 000 individual structures have been characterized. They include sterols which are constituents of eukaryotic membranes, steroid hormones, prenylquinones involved in electron transport during respiration and photosynthesis, sugar carriers for polysaccharide biosynthesis, carotenoids, prenylated proteins and prenylated derivatives of adenosine in tRNA, as well as a very large variety of compounds with less evident physiological roles. All these compounds are derived from isoprenoid diphosphates synthesized from the isomeric five-carbon intermediates isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Sacchettini & Poulter, 1997).

Isopentenyl diphosphate isomerase (IPP isomerase; E.C. 5.3.3.2) catalyzes a key step in the isoprenoid biosynthetic pathway by converting IPP to DMAPP, its highly electrophilic isomer. IPP then condenses with DMAPP. Addition of several IPP molecules to the resulting condensation product and reactions involving other molecules ultimately form the numerous isoprenoid structures found in nature (Poulter & Rilling, 1981). The isomerization of IPP to DMAPP is thought to proceed by a stereoselective antarafacial [1.3] protonation–deprotonation mechanism with a putative carbocation intermediate (Muehlbacher & Poulter, 1985, 1988; Reardon & Abeles, 1985, 1986). The stereochemistry of the reaction suggests that the active site contains two nucleophilic groups located on opposite faces of the allyl moiety of IPP to allow proton addition and elimination. IPP isomerases from various sources have a conserved cysteine residue in an N*xx*C*x*HP motif and a conserved glutamate residue (bold) in an E*x***E** motif (Hahn *et al.*, 1999). Experiments performed on *Saccharomyces cerevisiae* IPP isomerase involving active-site-directed irreversible inhibitors and site-directed mutagenesis have shown that these two conserved amino acids are required for isomerase activity (Street & Poulter, 1990; Street *et al.*, 1994). A better understanding of the catalytic mechanism will require structure determination of the enzyme. As the first step toward structure elucidation of IPP isomerase, we report here the overexpression, crystallization and preliminary X-ray diffraction studies of the *E. coli* IPP isomerase.

2. Methods and results

2.1. Cloning, overexpression and purification of *E. coli* IPP isomerase

The *idi* gene encoding the *E. coli* IPP isomerase (Hahn *et al.*, 1999) was amplified by the polymerase chain reaction (PCR) using *E. coli* XL1 Blue colonies (Stratagene) as template. The forward (5′-GGCATATG-CAAACGGAACACGTC-3′) and reverse (5′-GGCCTCGAGTTTAAGCTGGGTAAA-TGCAG-3′) oligonucleotide primers were designed using the published sequence (Blattner *et al.*, 1997; Wang *et al.*, 1999). The PCR product was inserted into *Nde*I/*Xho*I-digested pET-30b vector (Novagen), giving the pYL20 plasmid. The resulting protein, identified by DNA sequencing as IPP isomerase carrying a C-terminal histidine tag (LEHH-HHHH), was overexpressed in *E. coli* BL21(DE3)pLysS cells (Novagen).

To facilitate structure solution, a selenomethionyl (SeMet) IPP isomerase was produced using the method described by Doublié (1997). Expression of the recombinant enzyme was induced overnight at 288 K using 1 m*M* IPTG. Cells from 1 l of culture were resuspended in 50 m*M* Tris–HCl pH 7.4 and cell disruption was achieved by sonication for 10 min in a Raytheon sonic oscillator (250 W; 10 kHz). Debris was removed by centrifugation at 15 000*g* for 10 min. Purification was performed using nickel-affinity chromatography with resin (10 ml chelating Sepharose

supplied by Amersham Pharmacia) pre-equilibrated with 50 m*M* Tris–HCl pH 7.4. Bound protein was eluted using a 100 ml linear gradient of 0–0.5 *M* imidazole in 50 m*M* Tris–HCl pH 7.4. The fractions containing active IPP isomerase were pooled and dialyzed against Tris–HCl 50 m*M* pH 7.4, 0.2 m*M* EDTA, 3 m*M* β-mercaptoethanol. Fractions were analyzed by SDS–PAGE and *in vitro* enzyme activity assay (Ramos-Valdivia *et al.*, 1997). The production of the SeMet enzyme was 70 mg per litre of culture (Fig. 1). Incorporation of SeMet was confirmed by mass spectrometry. The SeMet protein presents a molecular weight of 21 760 Da, corresponding to the MW of the wild-type enzyme (21 572 Da) plus 188 Da. This indicates the presence of four selenomethionines.

2.2. Crystallization of selenomethionyl *E. coli* IPP isomerase

The purified enzyme samples were concentrated to about 7 mg ml^{-1} by ultrafiltration (YM10, Amicon) and the protein concentration was estimated by UV absorption (Kalckar & Shafran, 1947). Crystallization trials were performed by the hanging-drop vapour-diffusion method (McPherson, 1982) using 24-well tissue-culture VDX plates (Hampton Research) at 293 K. Each drop was prepared by mixing 2 µl of protein solution with the same volume of reservoir solution. The drops were suspended over 0.6 ml of reservoir solution. Initial searches for crystallization conditions were performed using the standard sparse-matrix crystal screens (Jancarik & Kim, 1991) from Hampton Research (Crystal Screen I and Crystal Screen II) and the usual precipitating agents. Crystals of the SeMet IPP isomerase were obtained after 4 d in 10%(*w*/*v*) PEG 2000 monomethylether, 100 m*M* Tris/maleate buffer at pH 5.5 in the presence of 100 m*M* ammonium sulfate. Their approximate dimensions are 0.2 × 0.1 × 0.1 mm (Fig. 2).

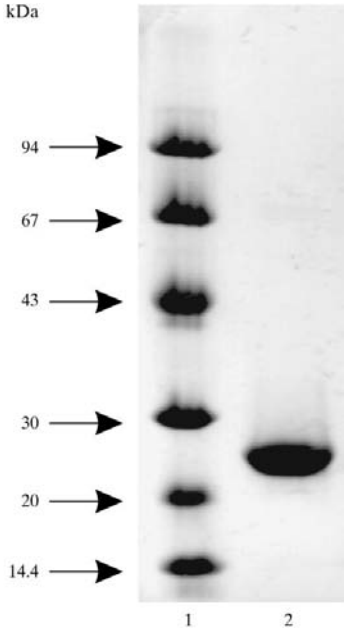

Figure 1
Polyacrylamide gel electrophoresis under denaturing conditions of the purified *E. coli* selenomethionyl IPP isomerase. Lane 1, molecular-weight markers (Amersham-Pharmacia). Lane 2, purified enzyme.

2.3. X-ray diffraction experiments and analysis

Crystals of the SeMet IPP isomerase were tested at the European Synchrotron Radiation facility (ESRF) in Grenoble. A 98.1% complete data set has been measured to 1.45 Å resolution on beamline ID14-EH2 using an ADSC-Q4 CCD-based detector, at a wavelength of 0.9326 Å. The crystal was first transferred for a few seconds into a cryoprotectant mixture containing the crystallization solution [10%(*w*/*v*) PEG 2000 monomethylether, 100 m*M* Tris/maleate pH 5.5, 100 m*M* ammonium sulfate] to which 25% of glycerol was added. The crystal was subsequently flash-cooled in liquid nitrogen and maintained at 100 K during data collection. It displays trigonal symmetry, with unit-cell parameters $a = b = 71.3$, $c = 61.7$ Å. Characteristic systematic absences and scaling statistics indicate $P3_121$ or $P3_221$ symmetry. Assuming a single IPP isomerase monomer in the asymmetric unit, the calculated Matthews coefficient V_M (Matthews, 1968) is 2.4 Å^3 Da^{-1} with 48.4% solvent content. The redundancy and R_{merge} (all data) values are 8.7 and 6.9%, respectively. The structure determination of IPP isomerase by the multi-wavelength anomalous diffraction (MAD) technique is currently in progress.

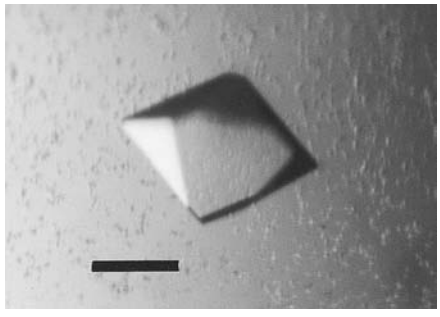

Figure 2
Crystal of selenomethionyl IPP isomerase obtained by the hanging-drop vapour-diffusion technique. The scale bar corresponds to 0.1 mm.

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