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Crystallographic analysis of enzymes involved in terpenoid biosynthesis

Takeo TOMITA, Kenichi MATSUDA, Taro OZAKI, Makoto NISHIYAMA, and Tomohisa KUZUYAMA*
Biotechnology Research Center, The University of Tokyo, Tokyo 113-8657, Japan

Introduction

Terpenoids have been an important resource for biologically active compounds because of their structural diversity (Maimone & Baran, 2007, Sacchettini & Poulter, 1997). Over the past decades, various studies have been performed to identify the biosynthetic mechanism for the terpenoid complexity. The complexity of the terpenoid skeleton is generated by the condensation of C5 isoprene units and subsequent cyclization. Usually, these condensation and cyclization reactions are independently catalyzed by isoprenyl diphosphate synthase (IDS) and cyclase, respectively.

Recently, we have identified and characterized CLDP synthase (CLDS) from a soil bacterium *Streptomyces* sp. CL190, which produces lavanducyanin, a phenazine with an *N*-linked cyclolavandulyl structure (Ozaki *et al.*, 2014). CLDS catalyzes both the condensation of two molecules of C_5 dimethylallyl diphosphate (DMAPP) and subsequent cyclization to form CLDP and we have proposed a likely reaction mechanism for CLDS (Scheme 1). CLDS belongs to *cis*-isoprenyltransferase. This enzyme family catalyzes the condensation of DMAPP to form compounds with polyprenyl chains. Among this family, undecaprenyl diphosphate synthase (UDS) catalyzes the cis-prenyl chain elongation onto trans, trans-farnesyl diphosphate (FPP) to produce undecaprenyl diphosphate (UPP), which is indispensable for the biosynthesis of bacterial cell wall. The crystal structure of the enzyme from *Escherichia coli* and *Micrococcus luteus* were determined and the structural basis of the condensation reaction is clarified. CLDS is unique since CLDS catalyzes not only condensation but also cyclization of the intermediate, while CLDS and UDS share conserved amino acid residues that recognize the phosphate moiety of the prenyl diphosphate substrate.

In the present study, to gain insight into structural basis of this unusual CLDS-catalyzed two step reaction, we crystallized CLDS and then we collected and analyzed X-ray diffraction data from the crystals of CLDS.

Materials and Methods

2.1. Protein expression and purification

E. coli BL21-CodonPlus(DE3)-RIL cells possessing pHIS8-CLDS-NH or pET-CLDS-CH, those are plasmids for expression of CLDS fused with histidine tag at the N- or C- terminal, respectively, were grown in 2 x YT broth in the presence of kanamycin (50 μg/ml) and chloramphenicol (30 μg/ml) at 303 K. The gene expression was induced by adding 0.1 mM isopropyl β-D-thiogalactopyranoside and the culture was continued for additional 12 -14 h. The samples were purified with Ni^{2+}-resin column chromatography, and gel filtration column chromatography. Based on the elution volume from the size exclusion column, the oligomeric state of the protein was estimated to be a dimer. The homogeneity over 95 % of the purified CLDS were verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). CLDS-NH and CLDS-CH of over 40 mg were purified.

2.2 Crystallization

For CLDS-NH in the presence of DMAPP and $MgSO_4$, crystallization conditions were screened by the hanging-drop vapor-diffusion method using Crystal Screen kits (Hampton Research), and WizardScreen kits (Emerald Biosystems). Drops of 2 μl consisting of 1 μl reservior fluid and 1 μl of 10 mg/ml CLDS solution with and without 5 mM DMAPP and 2 mM $MgSO_4$. A few crystal were obtained from a droplet using solution containing 100 mM HEPES-NaOH (pH7.0), 24 % PEG 2000 in the presence of DMAPP and $MgSO_4$ (Figure 1A). Crystals were used for X-ray diffraction.

For CLDS-CH, crystallization conditions were screened by the hanging-drop vapor-diffusion method using Crystal Screen kits (Hampton Research), and WizardScreen kits (Emerald Biosystems). Drops of 2 μl consisting of 1 μl reservior fluid and 1 μl of 10 mg/ml CLDS solution with and without 5 mM DMAPP and 2 mM $MgSO_4$. A few crystal were obtained from a droplet using solution No.31 (100 mM Tris-HCl(pH7.0), 1 M Sodium citrate tribasic, and 200 mM Sodium chloride) of Wizard II in the absence of DMAPP and $MgSO_4$ (Figure 1B). Crystals were used for X-ray diffraction.

2.3 Data collection and processing

Prior to data collection, crystal was directly flash-cooled in a nitrogen gas stream at 95 K. Diffraction data (λ = 1.0 Å) were collected by a charge-coupled device (CCD) camera (ADSC Quantum 315r) under the following conditions; oscillation angle, 1.0 degree; number of images collected, 180; exposure time, 0.9 s, at the 5A station of the Photon Factory, High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. Diffraction images were indexed and scaled with HKL2000.

* utkuz@mail.ecc.u-tokyo.ac.jp