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Crystallographic analysis of enzymes involved in the biosynthesis of natural products possessing complicated chemical structures

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1 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. 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. 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 proposed. 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.

2 Experiment

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 IPTG and the culture was continued for additional 12-14 h. The samples were purified with Ni^{2+}-resin column chromatography, and Superdex 75 gel filtration column chromatography. The homogeneity over 95 % of the purified CLDS were verified by SDS-PAGE. CLDS-NH and CLDS-CH of over 40 mg were purified.

Crystallization

Crystallization conditions were screened by the hanging-drop vapor-diffusion method using Crystal Screen (Hampton Research) and the Wizard crystallization screen series (Emerald Bio). The screenings were set up using 2 μl drops consisting of 1 μl reservoir solution and 1 μl 10 mg ml^{-1} CLDS solution with and without 5 mM DMAPP and 2 mM $MgSO_4$. We also performed screening using CLDS solution containing 5 mM DMSPP, which is an analog of DMAPP, and 2 mM $MgSO_4$ to capture pseudo-pre-reaction state of CLDS.

3 Results and Discussion

We determined crystal structure of CLDS by the single-wavelength anomalous diffraction method with a SeMet-substituted protein (Fig. 1) [1]. CLDS adopts typical fold for cis-prenyl synthases and forms a homo-dimeric structure. PPi formed as a by-product of the CLDS reaction and remained at the active site with Mg^{2+} ion and Tris because we used the buffer containing Tris, DMAPP, and $MgSO_4$ in the crystallization mixture. An in vitro reaction using a regio-specifically 2H-substituted DMAPP substrate revealed the intramolecular proton transfer mechanism of the CLDS reaction. The CLDS structure and structure-based mutagenesis provide mechanistic insights into this unprecedented terpene synthase [1-2].

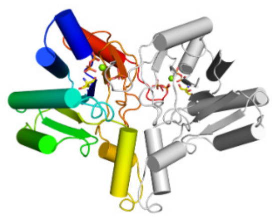

Fig. 1: Crystal structure of CLDS

References

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