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 C5 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 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 Mg²⁺ ion and Tris because we used the buffer containing Tris, DMAPP, and MgSO₄ 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].

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 Mg²⁺ ion and Tris because we used the buffer containing Tris, DMAPP, and MgSO₄ 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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