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Crystal structures of enzymes involved in the biosynthetic pathways using amino-group carrier protein, AmCP

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1 Introduction

The lysine biosynthetic pathway is classified into two types; the diaminopimelate (DAP) pathway in bacteria and plants, and the α-aminoadipate (AAA) pathway in fungi and yeast. We found that a bacterium, *Thermus thermophilus* synthesizes lysine via a novel AAA pathway using a small acidic protein called LysW. *T. thermophilus* uses LysW protein to protect α-amino group of AAA to avoid self-cyclization of the intermediate. Our previous studies including crystallographic analyses revealed that LysW functions not only as an amino-group protecting group but also as a carrier protein interacting with each enzyme electrostatically. Therefore, we hereafter call LysW as amino-group carrier protein (AmCP). Since our discovery of AmCP-mediated lysine biosynthesis through AAA in *T. thermophilus*, many microorganisms including thermophilic bacteria and archaea synthesize lysine and arginine by a similar pathway, indicating that this pathway is one of evolutionary origins of the amino acid biosynthetic pathways.

Furthermore, we have found that *Streptomyces* uses AmCP in the biosynthesis of secondary metabolites which containing non-proteinogenic amino acid (2*S*,6*R*)-diamino-(5*R*,7)-dehydroxy-heptanoic acid, DADH [1]. DADH is incorporated into a novel peptide metabolite, vazabitide A, featuring an azabicyclo-ring structure, by nonribosomal peptide synthetases and subsequent modification enzymes. The biosynthetic gene clusters of azinomycin B and ficellomycin, which also possess azabicyclo-ring moiety, contain DADH biosynthetic genes including AmCP gene, suggesting that these compounds are also biosynthesized via AmCP. Since an aziridine ring in this azabicyclo-ring is the key moiety for DNA alkylation, we are interested in the mechanism of this ring-formation.

Taken together, the elucidation of the structural bases of this unprecedented biosynthetic pathway is important for the further understanding of AmCP-mediated amino acid biosynthetic machinery. Here we present the structural analysis of the enzymes involved in the DADH biosynthesis and aziridine-ring formation.

2 Experiment

Purification of recombinant proteins

Vzb10/11 and AziU3/U2 complex, which are enzymes involved in aziridine ring formation in biosynthesis of vazabitide A and azinomycin B, respectively, and Fic25 involved in the DADH biosynthesis in ficellomycin biosynthesis, were overexpressed in *E. coli* Rosetta2(DE3) and BL21-Codon-Plus (DE3)-RIL as hosts. From cell lysates prepared by sonication, these proteins were purified using Ni^{2+}-NTA column, followed by gel filtration chromatography (Superdex 200) for crystallization.

Crystallization

The crystallization conditions for each protein (protein complex) were screened with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION screen by hanging drop vapor diffusion method at 20ºC. Optimization of the crystallization conditions were also performed by changing the concentration of precipitants and pH.

3 Results and Discussion

Structure of AziU2/U3 complex

We successfully determined the structure of AziU2/U3 complex at 1.75 Å resolution by SAD method. The complex forms hetero-tetramer with a novel fold. We also determined the structure of AziU2/U3 in complex with its substrate at 1.8 Å resolution. The structural information and the activity assay using variants suggested the catalytic mechanism for unprecedented aziridine-ring formation [2].

Structure of Fic25

We crystallized Fic25, which is an aminotransferase involved in the production of DADH in the ficellomycin biosynthesis, and successfully determined the structure in apo, holo-forms and reaction intermediate-bound form at 1.80, 1.93, and 1.82 Å resolution, respectively. DADHs biosynthesized in ficellomycin and vazabitide A have different stereochemistry. The structure with PLP-substrate adduct and the activity assay using variants of Fic25 and Vzb9, which is a homologous protein in vazabitide A biosynthesis, revealed the mechanism to synthesize stereoisomers of DADH [3].

References

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