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Crystal structures of enzymes involved in novel lysine biosynthetic pathway using amino-group carrier protein

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1 Introduction

Lysine biosynthetic pathway is classified into two types; diaminopimelate (DAP) pathway in bacteria and plants, and α-aminoadipate (AAA) pathway in fungi and yeast. We previously found that a bacterium, *Thermus thermophilus* synthesizes lysine via a novel type of AAA pathway using an amino-group carrier protein called LysW. Although the enzymes involved in the first half of the pathway in *T. thermophilus* are similar to those of fungi, the latter part is composed of the enzymes similar to those in arginine biosynthesis. Unlike in the arginine biosynthesis, *T. thermophilus* uses LysW protein to protect α-amino group of AAA. LysW is highly acidic protein and our previous studies including crystallographic analyses revealed that LysW functions not only as an amino-group protecting group but also as a carrier protein by interacting with each enzyme through the electrostatic interactions. Therefore, we hereafter call LysW as amino-group carrier protein (AmCP). Since our discovery of AmCP-mediated lysine biosynthesis through AAA in *T. thermophilus*, evidences have mounted that many microorganisms including thermophilic bacteria and archaea synthesize lysine and also arginine by a similar pathway, indicating that this pathway is one of evolutionary origins of the amino acid biosynthetic pathways.

Recently, we found that a kind of *Streptomyces* used 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 successive modification enzymes.

We also found that AmCP was also used for the biosynthesis of a compound, s56-p1, produced by another strain of *Streptomyces* [2]. s56-p1 contains a unique nitrogen-nitrogen bond (hydrazine moiety) fused with the DADH-derivative moiety. We identified the unprecedented machinery for hydrazine moiety in the strain and also found this machinery is widespread across several bacterial phyla, highlighting the overlooked potential of bacteria to synthesize hydrazine.

Thus, to reveal the structural bases of these unprecedented biosynthetic pathway is important for the further understanding of AmCP-mediated amino acid biosynthetic machinery. Here, we describe the trial to determine the crystal structures of enzymes involved in these pathways to reveal the structural basis of this unprecedented biosynthetic pathway including AmCP.

2 Experiment

Purification of recombinant proteins

Complex of OrfX and OrfY which are enzymes involved in biosynthesis of vazabitide A, and OrfZ which is an enzyme involved in biosynthesis of s56-p1 were overexpressed in *E. coli* Rosetta2 (DE3) and *E. coli* BL21-Codon-Plus (DE3)-RIL as the host, respectively. From cell lysates prepared by sonication, Complex of OrfX and OrfY were purified through Ni^{2+}-NTA column and OrfZ was purified through strep-tactin column, following by gel filtration chromatography (Superdex 200) for crystallization.

Crystallization of complex of OrfX and OrfY, OrfZ, and OrfA

The condition for crystallization of Complex of OrfX/Y, OrfZ, and OrfA were screened with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method at 20 ℃. Optimization of the crystallization condition was also conducted by modifying the concentration and pH.

3 Results and Discussion

X-ray diffraction analyses of complex of OrfX and OrfY

We found the crystallization condition of complex of OrfX and OrfY. We carried out X-ray diffraction analyses, and obtained data set at 1.9 Å resolution. The proteins did not show any similarity with structural known proteins, we determined the structure of them by means of single-wavelength anomalous dispersion (SAD) method at 1.8 Å resolution. The analysis revealed that they took a new protein fold. Furthermore, we successfully determined the structure in the substrate-bound form, which suggested the catalytic mechanism for unprecedented reaction.

X-ray diffraction analysis of OrfZ

We have not found the crystallization condition of OrfZ. Crystallization of its homologous enzyme and is now in progress.

X-ray diffraction analysis of OrfA

We found several crystallization condition of OrfA. X-ray diffraction analyses gave the data set at 1.8 Å resolution and we successfully determined the structure by using molecular replacement method. We are currently trying the co-crystallization of OrfA with its substrate analog.

References

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