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Structural analysis 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. Previously, 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 studies including crystallographic analyses revealed that LysW functions not only as a protecting group of an amino group of the substrate/intermediates 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 found that *Streptomyces* uses AmCP in the biosynthesis of secondary metabolites which containing non-proteinogenic amino acid (2S,6R)-diamino-(5R,7)-dehydroxy-heptanoic acid, DADH [1]. DADH is incorporated into a novel peptide metabolite, vazabitide A with an azabicyclo-ring structure. The biosynthetic gene clusters of azinomycin B and ficellomycin, which also possess azabicyclo-ring moiety, contain DADH biosynthetic genes including AmCP gene. Our previous studies about aminotransferases involved in DADH biosynthesis revealed that aminotransferases have a key role in stereoisomers of DADH [2]. Aminotransferase is conserved and functions in AmCP-mediated biosynthesis discovered so far. In this study, we determined crystal structures of two aminotransferases in different AmCP-mediated biosynthesis.

2 Experiment

Purification of recombinant proteins

Our previous studies have elucidated the recognition mechanism of LysW by lysine biosynthetic enzymes by the crystal structure with mutational analysis; however, how LysJ, an aminotransferase, recognizes LysW remains unknown. LysJ and LysW from *T. thermophilus* were overexpressed in *E. coli* BL21-Codon-Plus(DE3)-RIL as a host. These proteins were purified from cell lysates prepared by sonication and heat treatment using Ni^{2+}-NTA column or anion-exchange column, followed by gel filtration chromatography for crystallization.

Genes encoding heterooligomeric aminotransferase were found in AmCP-containing gene cluster from *Serratia*. We prepared the recombinant proteins of this heterooligomeric aminotransferase using *E. coli* BL21(DE3) as a host. The purification was conducted by the affinity chromatography and gel-filtration chromatography using Ni2+-NTA column and Superdex200, respectively.

Crystallization

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

3 Results and Discussion

Crystal structure of LysJ·LysW complex

We could determine the crystal structure of LysJ in complex with LysW at 2.40 Å resolution. Crystal structure revealed that LysJ recognizes LysW with negatively charged surface electrostatically as seen in other biosynthetic enzymes. LysW consists of a globular domain and C-terminal extension domain to which the substrate/intermediates are attached. Since we could not observe the electron densities for the C-terminal extension LysW in the crystal structure, we are now trying to crystallize LysJ with a synthetic peptide to elucidate how the C-terminal extension is recognized by LysJ.

Crystal structure of heterooligomeric aminotransferase

We successfully determined the crystal structure of aminotransferase from *Serratia* at 2.31 Å resolution, although its function in the AmCP-mediated biosynthesis is still unknown. To the best of our

knowledge, the structure reveals that it is a heterodimeric aminotransferase composed of two different subunits, which has never been known before.

References

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