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Structural analysis of aminotransferases involved in the biosynthetic pathways mediated by amino-group carrier protein, AmCP

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1 Introduction

The amino-group carrier protein (AmCP) is a unique carrier protein identified in the novel lysine biosynthetic pathway via α-aminoadipate (AAA) in the hyperthermophilic bacterium *Thermus thermophilus* [1]. In this system, the substrate is directly conjugated via its α-amino group to the γ-carboxy group of C-terminal Glu residue of AmCP without any post-translational modification. Our previous structural studies revealed that AmCP acts not only as a protecting group for the amino group of the substrate and intermediates, but also as a carrier protein that interacts electrostatically with biosynthetic enzymes. To date, all enzyme·AmCP complex structures have been elucidated except that of the aminotransferase LysJ.

Since our discovery of the AmCP-mediated lysine biosynthesis pathway, similar lysine and arginine (ornithine) biosynthetic routes have been identified in thermophilic bacteria and archaea, suggesting that this mechanism represents an evolutionary origin of amino acid biosynthesis.

Furthermore, we showed that AmCP is also involved in the biosynthesis of secondary metabolites in *Streptomyces*. A novel non-proteinogenic amino acid, (2*S*,6*R*)-diamino-(5*R*,7)-dehydroxy-heptanoic acid (DADH), is synthesized via an AmCP-mediated pathway and incorporated into natural products containing an azabicyclo-ring [2]. We also found that aminotransferases in DADH pathway determine its stereochemistry [3]. In addition, we identified *amcp*-containing biosynthetic gene clusters in various bacteria, different from lysine or DADH biosynthesis, which are likely involved in the production of other amino acid-like compounds. In this study, we conducted structural analyses of LysJ and aminotransferase from one of these clusters to gain insights into AmCP-mediated biosynthetic mechanisms.

2 Experiment

Crystallization of LysJ·AmCP complex

To determine the structure of AmCP·LysJ complex, the recombinant proteins of LysJ and AmCP produced in *E. coli* were purified. To visualize the interaction of the C-terminal arm of AmCP, an adduct of PLP and synthetic peptide corresponding to the C-terminal arm was co-crystallized with LysJ.

Crystallization of heterooligomeric aminotransferase

We found an *amcp*-containing gene cluster in *Serratia*, different from known lysine or DADH biosynthetic gene cluster. A notable feature of this cluster is the presence of a full-length aminotransferase gene and a truncated gene. These two proteins were co-produced in *E. coli*, purified as a heterooligomer, and used for crystallization.

3 Results and Discussion

Crystal structure of LysJ·AmCP complex

We determined the crystal structure of LysJ·AmCP complex and LysJ·PLP-Lys adduct at 2.4 Å and 1.9 Å resolution, respectively. Although the C-terminal arm of AmCP was not visible in LysJ·AmCP structure, its position was inferred by AlphaFold model. The Lys moiety of PLP-Lys adduct locates near the C-terminal Glu residue in AlphaFold model, indicating the model is plausible.

Crystal structure of the heterooligomeric aminotransferase from Serratia

The aminotransferase from *Serratia* forms a heterooligomer consisting of two different subunits. We also determined the structures of this enzyme in complex with PMP and the PLP-Glu adduct. Enzyme assays using variants and MD simulations showed that heterooligomer formation is essential for enzymatic activity, since the active site is composed of the residues from both subunits. Surface charge analysis revealed a basic patch likely to interact with the acidic AmCP, suggesting a novel recognition mode distinct from that of LysJ or aminotransferases in DADH biosynthesis. These findings provide new insights into the biosynthetic mechanism involving AmCP in *Serratia*, expanding our understanding of the functional diversity of AmCP-mediated pathways [4].

References

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