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Thermus thermophilus のリジン生合成酵素 LysZ とキャリアタンパク質 LysW の構造機能解析

The crystal structures of amino acid kinase LysZ and carrier protein LysW involved in lysine biosynthesis of *Thermus thermophilus*

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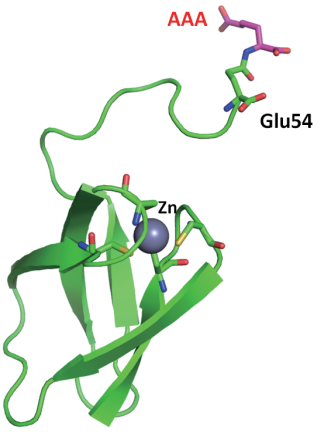
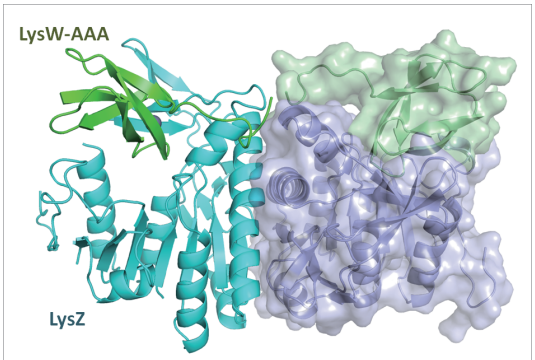
In bacteria and plants, it had generally been thought that lysine is produced *via* the diaminopimelate (DAP) pathway. However, we found that *Thermus thermophilus* synthesizes lysine *via* α-aminoadipate (AAA) like fungi and yeast [1]. Lysine biosynthesis through the AAA pathway can be devided into two parts: one from 2-oxoglutarate to AAA and the other from AAA to lysine. Although the AAA pathway in lower eukaryotes proceeds with saccharopine as a biosynthetic intermediate in the latter half of the pathway, lysine synthesis of *T. thermophilus* is unique. The latter half of the pathway in *T. thermophilus* does not include saccharopine as an intermediate but proceeds in a manner similar to that of arginine biosynthetic pathway. Arginine biosynthetic pathway starts with transacetylation by protecting α-amino group of glutamate with the acetyl group. On the other hand, LysX, the enzyme that catalyzes the first step of the latter pathway of lysine biosynthesis, does not exhibit similarity to ArgA, but protects the α-amino group of AAA by attachment to the γ-carboxyl group of the C-terminal Glu54 of a small protein named LysW, of only 54 amino acid residues [2]. LysW is composed of many acidic amino acids, while enzymes involved in the latter half of the pathway possess the active sites surrounded by highly positively charged amino acid residues, suggesting that acidic globular domain of LysW can interact with enzymes in the pathway electrostatically. These facts allow us to propose that LysW acts as a “carrier protein” for the efficient biosynthesis of lysine. In this study, to elucidate the function of LysW as a “carrier protein”, we determined the crystal structures of LysW and the complex with LysZ, which catalyzes the second step in the latter half of the lysine biosynthetic pathway; phosphorylation of the δ-carboxyl group of the AAA moiety of LysW-γ-AAA that is generated by the LysX reaction, with ATP.

We prepared LysW-γ-AAA by *in vitro* LysX reaction, and conducted the crystallization screening in the presence of LysZ. Using the crystals showing good diffraction pattern, we determined the crystal structures of LysW-γ-AAA and the complex of LysW and LysZ at 1.20 Å and 1.85 Å resolution, respectively. The crystal structure of LysW-γ-AAA reveals that LysW is monomeric and the amino group of AAA is actually protected by the γ-carboxyl group of Glu54 from LysW. In addition, the electrostatic potential calculated with the crystal structure of LysW-γ-AAA indicates that molecular surface of LysW is covered with negative charge as expected. The crystal structure of



Crystal structure of LysW-γ-AAA

LysZ-LysW complex elucidates that LysZ forms a dimer and LysW is bound to LysZ at one to one ratio. The interaction between LysZ and LysW involves ionic bonds between arginine and lysine residues from LysZ and glutamate and aspartate residues from LysW, and several hydrogen bonds. The surface charge distributions of LysZ and LysW suggest that the positively charged surface of LysZ is covered by negatively charged LysW. This is consistent with the analysis with ITC (isothermal titration calorimetry) that showed that the binding of LysW to LysZ is driven by enthalpy force. In this study, we have verified the function of LysW as a "carrier protein" to assure the efficient biosynthesis. In this presentation, we will also report about the enzymatic analysis using mutants carrying mutations at the interaction sites between LysZ and LysW.



Crystal structure of LysZ-LysW complex

Reference

[1] Aspartate kinase-independent lysine synthesis in an extremely thermophilic bacterium *Thermus thermophilus*: lysine is synthesized via α-aminoadipic acid, not via diaminopimelic acid. Kobashi, N. *et al. J. Bacteriol.* (1999) 181, 1713-1718

[2] Discovery of proteinaceous N-modification in lysine biosynthesis of *Thermus thermophilus*. Horie, A. *et al. Nat. Chem. Biol.* (2009) 5, 673-679