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

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Introduction

Lysine biosynthetic pathway is classified into two groups; diaminopimelate (DAP) pathway in bacteria and plants, and α-aminoadipate (AAA) pathway in fungi and yeast. We previously found that a bacterium, *Thermus thermophilus* synthesize 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 electrostatically. Since our discovery of LysW-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 origins of the amino acid biosynthetic pathways. Thus, to reveal the structural basis of this novel lysine biosynthetic pathway is important for the further understanding of LysW-mediated amino acid biosynthetic machinery.

To date, we have clarified the electrostatic interaction between LysW and several enzymes involved in the AAA pathway. Here we report the structural analysis about LysK, which catalyzes the final step of this pathway. LysK is a member of M20 metallopeptidase family, which catalyzes the release of lysine from LysW-γ-Lys, which produced by enzyme reactions from AAA, in the presence of metal ions. Although some structures of M20 family peptidases are determined, most of those structures are determined in the absence of substrates. Therefore, structural determinants of substrate specificity of M20 family peptidases are not known due to the lack of structural information. As seen in other biosynthetic enzymes in AAA pathway, the active site of LysK was also predicted to be surrounded by positively-charged residues to recognize the negatively-charged globular domain of LysW. However, the experimental verification by the crystal structure has not yet been performed until now. To clarify the substrate recognition manner and the interaction mechanism between LysK and LysW-γ-Lys, we performed crystallographic analysis of LysK and LysW complex from *T. thermophilus* HB27.

Materials and Methods

Purification of recombinant proteins

LysK was overexpressed in *Escherichia coli* BL21-Codon-Plus (DE3)-RIL as a host using 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) for induction. From cell lysate prepared by sonication, LysK was purified through heat treatment, Strep-Tactin affinity chromatography, and Superdex 200 gel filtration chromatography.

LysW was overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as a host using 0.1 mM IPTG for induction. Cells were harvested and sonicated to prepare the lysate. LysW was purified through heat treatment, DE52 anion exchange chromatography, and Superdex 30 gel filtration chromatography.

Crystallization of LysK/LysW complex

Crystallization condition for LysK/LysW co-crystallization was screened in the presence of lysine using Crystal screen I and II, and Wizard classic I, II, and III by hanging drop vapor diffusion method.

Results and Discussion

Through heat treatment and successive column chromatographies, we could purify LysK and LysW.

We found the crystallization condition in which ammonium sulfate was used as a precipitant in the presence of LysW and lysine. We collected the diffraction data at 2.4 Å resolusion and determined crystal structure of LysK/lysine complex. Although we added LysW and lysine to the mixture, only lysine was bound to LysK. Although LysK did not bind metal ions since the crystallization condition did not contain any metals, the structure revealed that LysK has metal-binding site composed of same residues as other members of M20 metallopeptidase family. The structure also revealed lysine-binding manner of LysK and suggested structural determinants of substrate specificity of M20 family enzymes. The surface region surrounding the active site of LysK was occupied by positively charged residues, suggesting that LysK recognizes globular domain of LysW through electrostatic interaction. We are now trying to crystallize LysK/LysW-γ-Lys complex to exemplify the interaction manner between two proteins.

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