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

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Introduction

Bacteria and plants were thought to biosynthesize lysine from aspartate via the diaminopimelate pathway, while fungi biosynthesize lysine from α-ketoglutarate via α-aminoadipate (AAA), which is known as the AAA pathway. We previously found that a bacterium, *Thermus thermophilus* synthesize lysine via a novel type of AAA pathway. The enzymes involved in the first half of the pathway in *T. thermophilus* are similar to those of fungi. In the latter half of the AAA pathway in *T. thermophilus*, small acidic protein called LysW is responsible for protection of α-amino group of AAA and its derivatives unlike fungal AAA pathway. Since our discovery of LysW-mediated lysine biosynthesis through AAA, evidences have mounted that many microorganisms including thermophilic bacteria and archaea synthesize lysine by a similar pathway, indicating that this pathway is one of origins of lysine biosynthesis. Our study also suggested that LysW and its homologs act as a carrier protein to facilitate recognition of biosynthetic intermediates by the catabolic enzymes through electrostatic interactions.

LysY is the seventh enzyme involved in the AAA pathway in *T. thermoiphilus* catalyzing reversible reduction of LysW-γ-alpha-aminoadipic phosphate to LysW-γ-alpha-aminoadipic semialdehyde (LysW-γ-AASA). Amino acid sequence of LysY shows similarity to those of ArgC involved in arginine biosynthesis. Although these two enzymes catalyze similar reaction, ArgC does not utilize carrier protein as it catalyzes reversible reduction of *N*-acetyl-γ-glutamyl-phosphate to *N*-acetyl-γ-glutamic semialdehyde.

To date, apo structure of LysY from *T. thermophilus* HB8 has already been reported, whereas, it was not sufficient to understand interaction between LysY and LysW derivatives. To clarify the interaction mechanism of LysY and LysW, we performed crystallographic analysis of LysY and LysW-AASA complex from *T. thermophilus* HB27.

Materials and Methods

Purification of recombinant proteins

LysY was overexpressed in *E. 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, LysY was purified through heat treatment, Blue-Sepharose CL-6B affinity chromatography, Resource Q anion exchange 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. From cell lysate prepared by sonication, LysW was purified through heat treatment, DE52 anion exchange chromatography, and Superdex 75 gel filtration chromatography.

LysX and LysZ were overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as a host using 0.1 mM IPTG for induction. Both enzymes were prepared as C-terminal His_6-tagged proteins, then purified by heat treatment and Ni^{2+}-NTA affinity chromatography.

Preparation of LysW-AASA

Reaction mixture (10ml) containing 100 mM Tris-HCl, pH 8.5, 2 mM $MgSO_4$, 10 mM NADPH, 10 mM AAA, 20 mM ATP, 0.5 mg ml^{-1} LysX-His_6, 0.4 mg ml^{-1} LysZ-His_6, 0.3 mg ml^{-1} LysY, 2.5 mg ml^{-1} LysW was incubated for 2 hours at 65 °C. LysW-AASA was purified through Resource Q anion exchange chromatography and Superdex 30 gel filtration chromatography.

Crystallization of LysY/LysW-γ-AASA complex

Optimum condition for LysY/LysW-γ-AASA co-crystallization was initially screened with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method. The crystallization conditions were further optimized by changing pH and concentration of precipitant.

Results and Discussion

Through heat treatment and successive column chromatographies, we purified LysY, LysW, LysX-His_6, and LysZ-His_6, respectively. To prepare a substrate for LysY, we performed *in vitro* reaction using these proteins and AAA as a starting substrate in presence of Mg^{2+}, ATP, and NADPH. Formation of LysW-AASA was confirmed by mobility shift on Tricine-PAGE comparing to those of LysW and other LysW derivatives.

We found LysY/LysW-AASA co-crystallization condition in which polyethylene glycol was used as a precipitant. We determined crystal structure of LysY/LysW-γ-AASA complex with 1.8 Å resolution. The structure clarified electrostatic interaction between globular domain of LysW-AASA and LysY while C-terminal extention loop of LysW-γ-AASA was disordered. We are now trying to find another condition for LysY/LysW derivatives co-crystallization to determine structure of C-terminal region of LysW in complex with LysY.

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