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

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1 Introduction

Lysine biosynthetic pathway is classified into two types; diaminopimelate (DAP) pathway in bacteria and plants, and α-aminoadipate (AAA) pathway in fungi and yeast. We previously found that a bacterium, *Thermus thermophilus* synthesizes 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 through the electrostatic interactions. 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 lysine biosynthetic pathway is important for the further understanding of LysW-mediated amino acid biosynthetic machinery.

We have determined the crystal structures of the biosynthetic enzymes (LysX, LysZ, and LysY) in complex with LysW or its derivatives, whereas the crystal structures of LysJ and LysK, which catalyze the last two steps, in complex with LysW have not been determined. LysJ functions as aminotransferase to produce LysW-γ-Lys, and LysK is a metalloprotease hydrolyzing LysW-γ-Lys to release lysine. Previously, we determined the crystal structure of LysK·lysine complex. To understand the recognition mechanism of these enzymes for LysW derivatives, we performed the crystallization screening for both enzymes and the mutational studies for LysK.

2 Materials and Methods

Purification of recombinant proteins

LysJ and LysK and its mutants were overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as the hosts. From cell lysate prepared by sonication, LysJ was puridied through heat treatment, Ni^{2+}-NTA column, and gel filtration chromatography. LysK was purified through heat treatment, Strep-tactin column, and gel filtration chromatography.

LysW-γ-Lys was produced using *E. coli* BL21 (DE3) harboring the expression vectors for LysW, LysX, LysZ, LysY, and LysJ using 0.1 mM IPTG for induction supplemented with 5 mM AAA. From cell lysate prepared by sonication, LysW-γ-Lys was purified through heat treatment, anion exchange chromatographies, and gel filtration chromatography.

Activity assay for LysK mutants

To reveal the recognition mechanism for LysW in LysK, we introduced mutations for positively-charged residues around the active site and the residues consisting the cleft toward the active site. Activity of these mutants were measured using LysW-γ-Lys and CT10-γ-Lys, which is the synthetic oligopeptide containing the C-terminal 10 residues of LysW fused with lysine.

Co-crystalization of LysJ/LysW-γ-Lys

The condition for LysJ/LysW-γ-Lys co-crystalization was screened with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method.

3 Results and Discussion

Crystals of LysJ/LysW-γ-Lys

We found the crystallization condition of LysJ in which PEG3350 was used as a precipitant in the presence of LysW-γ-Lys. We analyzed the X-ray diffraction data, but it only diffracted at 5-6 Å resolution. Further optimization of the crystallization condition is necessary to obtain the data set.

Recognition mechanism of LysK for LysW-γ-Lys

The mutations at the cleft for the active site reduced the LysK activity, indicating that these residues well recognize the C-terminal extension domain and fused lysine. However, among the five positively-charged residues, only two mutants reduced their activity. Using CT10-γ-Lys as a substrate, the activity of these mutants was less decreased than the activity toward LysW-γ-Lys, suggesting that these residues are involved in the recognition of the globular domain of LysW. The docking model of LysK bound with LysW-γ-Lys suggested that a few specific interactions were observed between LysK and the globular domain of LysW. We assumed that the positively-charged area of LysK functions to attract LysW-γ-Lys near the active site, but weakly interact with the LysW derivative, as reported previously for other biosynthetic enzymes complexes with LysW.

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