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

Takeo TOMITA[1], Ayako YOSHIDA[1], Masanobu ARITA[1], and Makoto NISHIYAMA*[1]
[1]The Univ. of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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 evolutionary 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. For LysK, crystal structure in complex with lysine was determined and the mutational studies proposed the recognition mechanism of LysW by LysK. LysJ functions as aminotransferase to produce LysW-γ-Lys from LysW-γ-AAA semialdehyde in PLP-dependent manner. To understand the recognition mechanism of LysJ for LysW derivatives, we performed the crystallization screening for LysJ·LysW derivatives complex and the mutational studies for LysJ.

2 Materials and Methods

Purification of recombinant proteins

LysJ and its mutants was overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as a host. From cell lysate prepared by sonication, LysJ was puridied through heat treatment, Ni^{2+}-NTA column, and gel filtration chromatography (Superdex 200) for crystallization.

LysW-γ-Lys, which can be a substrate of LysJ, was produced using *E. coli* BL21 (DE3) harboring the expression vectors for LysW, LysX, LysZ, LysY, and LysJ with 5 mM AAA supplemented in the culture broth. From cell lysate prepared by sonication, LysW-γ-Lys was purified through heat treatment, anion exchange chromatography (DE52, monoQ), and gel filtration chromatography (Superdex 30). LysW was also prepared by the same procedure. LysW-γ-AAA was produced by the enzyme reaction by LysX using LysW and AAA as substrates, and purified in the same way as LysW.

Activity assay for LysJ and its mutants

To reveal the recognition mechanism for LysW-γ-Lys in LysJ, we introduced mutations for positively-charged residues around the active site, especially residues conserved in LysJ but not in ArgD, which is a paralogous protein in the general (not LysW-mediated) arginine biosynthesis. Activity of these mutants were measured using LysW-γ-Lys and *N*α-acetyllysine.

Co-crystalization of LysJ·LysW derivatives

The condition for co-crystallization of LysJ·LysW-γ-Lys or LysW derivatives was screened with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method at 20 ˚C.

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 or LysW. The optimization of the crystallization condition allowed the diffraction at 2.5-3 Å, however, the diffraction quality was not good enough to obtain the data set. Further optimization of the crystallization or cryo-protectant condition is necessary.

Recognition mechanism of LysJ for LysW-γ-Lys

Some of the mutations around the active site reduced the LysJ activity against LysW-γ-Lys but not against *N*α-acetyllysine. This indicated that these residues are not important for the catalysis but are involved in the recognition of LysW moiety of LysW-γ-Lys. These residues are located around the positively-charged surface of LysJ, suggesting that these residues might be responsible for the recognition of the globular domain of LysW.

* umanis@mail.ecc.u-tokyo.ac.jp