NW12A, NE3A, 5A, 17A/2015G552

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

Takeo TOMITA[1], Ayako YOSHIDA[1], Tetsu SHIMIZU[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 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.

LysY is the seventh enzyme involved in the AAA pathway of *T. thermoiphilus* catalyzing reversible reduction of LysW-γ- aminoadipic phosphate to LysW-γ-aminoadipic semialdehyde (LysW-AASA). We previously determined crystal structure of LysY from *T. thermophilus* HB27 complexed with LysW-AASA; however, the electron density of the C-terminal extension (Glu47-Glu54) of LysW-AASA was not visible in the crystal structure, indicating that the C-terminal extension of LysW-AASA is flexible. To capture the LysY·LysW complex with the C-terminal extension of LysW inserted into the active site of LysY, we herein performed crystallographic analysis using an inactive mutant of LysY (C148A) to stabilize the isopeptide-bonded substrate within the active site of LysY.

2 Materials and Methods

Purification of recombinant proteins

LysYC148A 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, LysYC148A was purified through heat treatment, Blue-Sepharose CL-6B affinity chromatography, Resource Q anion exchange chromatography, and Superdex 200 gel filtration.

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.

Preparation of LysW-γ-AAA

Reaction mixture (10ml) containing 100 mM Tris-HCl, pH 8.0, 2 mM $MgSO_4$, 10 mM AAA, 20 mM ATP, 0.5 mg ml^{-1} LysX-His_6 was incubated for 5 hours at 65 °C. LysW-γ-AAA was purified by Superdex 30 gel filtration.

Co-crystalization of LysY/LysW-AASA

Optimum condition for LysYC148A/LysW-γ-AAA co-crystalization was initially screened with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method. Crystallization conditions were further optimized by changing pH and concentration of precipitant.

3 Results and Discussion

We found the crystallization condition of LysYC148A in which PEG3350 was used as a precipitant in the presence of LysW-γ-AAA and $NADP^+$. We collected the diffraction data at 1.7 Å resolution and determined crystal structure of LysYC148A·LysW-γ-AAA·$NADP^+$ complex. In this crystal structure, the electron density for the C-terminal extension of LysW-γ-AAA was clearly observed, and its AAA moiety was bound to the active site of LysYC148A. A monomer of LysW-γ-AAA was recognized by two subunits of LysY forming a dimer, one subunit of which recognized the globular domain of LysW, whereas the other subunit accommodated the C-terminal extension, including the AAA moiety. The globular domain of LysW was recognized by a patch consisting of helix α9 and the β11-α10 loop. The extended LysW recognition loop and conserved arginine residue were identified as signatures to discriminate LysY from ArgC. Combined with the previously determined LysZ·LysW complex structure, LysW may simultaneously bind LysZ and LysY. These structural insights suggest the formation of a LysWZY ternary complex, in which the flexible C-terminal extension of LysW promotes the efficient transfer of the labile intermediate from the active site of LysZ to that of LysY during the sequential reactions catalyzed by LysZY.

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