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Crystal structures of the enzymes involved in lysine biosynthesis of *Thermus thermophilus*

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Introduction

Bacteria and plants were thought to biosynthesize lysine via the diaminopimelate pathway, while fungi biosynthesize lysine from α-aminoadipate (AAA). We preriously found that although it is a bacterium, *Thermus thermophilus* synthesized lysine via AAA. The enzymes involved in the first half of the pathway in *T. thermophilus* are similar to those involved in the leucine biosynthetic pathway or tricarboxylic acid cycle. However, the latter half of the pathway is totally different from the corresponding part of fungal biosynthetic pathway. Since our discovery of lysine biosynthesis through AAA, evidence has mounted that many microorganisms synthesize lysine by a similar pathway, indicating that this pathway is one of origins of lysine biosynthesis. To date, we have characterized the enzymes involved in this unique lysine biosynthetic pathway. To clarify the structure-function relationships of these enzymes, we performed crystallographic analyses of enzymes and proteins involved in lysine biosynthetic pathway. Here, we describe the newly determined crystal structures of TtLysW-AAA, TtLysW/TtLysZ complex, and LR5-1, which is a variant of homoisocitrate dehydrogeanse (HICDH) from *T. thermophilus*.

Materials and Methods

Data collection and processing.

The X-ray diffraction data of native proteins were collected using the beamline, NW12, NE3 and 5A at PF. The image sets were integrated and scaled using HKL2000.

Results and Discussion

TtLysW and TtLysW/TtLysZ complex

In *T. thermophilus*, α-amino group of AAA, which is an intermediate of lysine biosynthetic pathway, is protected by small acidic protein LysW. The first enzyme of the latter part of the pathway, LysX catalyzes the conjugation between α-aminogroup of AAA and γ-carboxyl group of C-terminal Glu54 in LysW to form LysW-γ-AAA. Recently, we have revealed that the globular domain of LysW is necessary for efficient synthesis of lysine. Therefore, LysW functions as not only a protecting group of AAA, but also a carrier protein to ensure the efficient synthesis of lysine. To elucidate the mechanism how LysW is recognized by lysine biosynthetic enzymes, we determined the crystal structures of LysW-γ-AAA and LysW/LysZ complex at 1.2 Å and 1.85 Å resolution, respectively. LysZ is the second enzyme in the latter part of the pathway, and catalyzes phosphorylation. The LysW-γ-AAA has highly negative charge. In the complex, positively charged region of LysZ is covered by negatively charged LysW. Thus, we could show that LysW actually functions as a novel carrier protein in the lysine biosynthetic pathway.

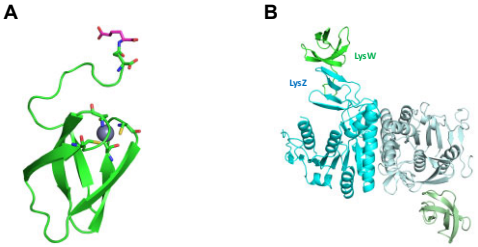

Fig.1 Structures of LysW-γ-AAA and LysW/LysZ complex. A. LysW-γ-AAA. B. LysW/LysZ complex.

LR5-1[1]

LR5-1 is one of the variants of TtHICDH with biological 3-isopropylmalate dehydrogenase (IPMDH) activity, which was found by directed evolution using a DNA-shuffling technique. LR5-1 possesses a 65-fold increased kcat/Km value for 3-IPM, compared with TtHICDH. To elucidate the mechanism of substrate recognition, we determined the crystal structure of LR5-1 at 2.4 Å resolution and revealed that I72, I84, and M85 on the helix α4 was displaced in a manner suitable for recognition of the hydrophobic γ-moiety of 3-IPM. (Fig. 2).

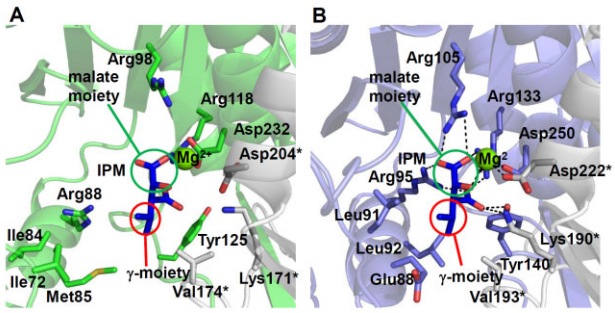

Fig. 2 Comparison of active sites between LR5-1 docked with 3-IPM/Mg^{2+} (A) and IPMDH from *Thiobacillus ferrooxidans* complexed with 3-IPM/Mg^{2+} (B).

Reference

1) Y. Suzuki *et al. Biochem. J.* 2010, 431(3):401-10.

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