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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 homocitrate synthase from *T. thermophilus* (TtHCS) and α-aminoadipate aminotransferase from *T. thermophilus* (TtAAA-AT).

Materials and Methods

Data collection and processing.

The X-ray diffraction data of native proteins were collected using the beamline, 5A, 6A, and NW-12 at PF. The image sets were integrated and scaled using HKL2000.

Results and Discussion

TtHCS[1]

TtHCS catalyzes the Aldol-type condensation of acetyl-CoA and α-ketoglutarate (α-KG) to synthesize homocitrate as the first step of lysine biosynthetic pathway through AAA. TtHCS is regulated via feedback inhibition by the end product, lysine, in competitive manner against α-KG. Here, we determined the crystal structure of TtHCS complexed with Co^{2+}/Lys at 1.80 Å resolution. In the complex, lysine is bound to the active site. Surprisingly, active site and inner region of TIM barrel take rearrangement to accommodate lysine, which has totally different chemical structure with α-KG (Fig. 1). This clearly revealed competitive inhibition mechanism of TtHCS. While the structure of TIM barrel domain is essentially the same between Co^{2+}/Lys and Cu^{2+}/α-KG. complexes, C-terminal small domain II is displaced, indicating that the domain is involved in allosteric mechanism. These findings not only lead the elucidation of a unique allosteric mechanism but also enable us to design new feedback resistant enzyme.

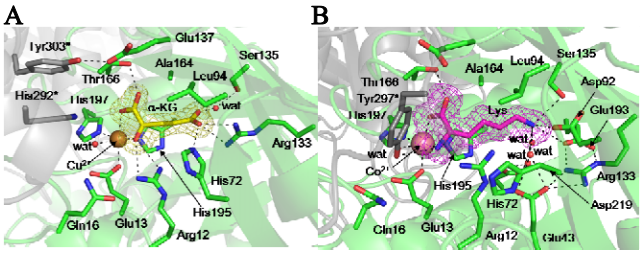

Fig.1 Active site structures of TtHCS.
A. Cu^{2+}/α-KG complex, B. Co^{2+}/Lys complex.

TtAAA-AT[2]

TtAAA-AT, a homolog of mammalian kynurenine aminotransferase II (Kat II), catalyzes the transfer of an amino group to 2-oxoadipate to yield AAA as fifth step of lysine biosynthesis in *T. thermophilus*. We have previously determined crystal structures of TtAAA-AT in four forms: PLP complex, PLP/Leu complex, PPL complex, and PPA complex. Here, we newly determined a crystal structure of TtAAA-AT complexed with PPE at 1.67 Å resolution. The crystal structure revealed that overall structure including the active site architecture was almost the same as that of the PPA complex. The bound PPE is recognized by a set of amino acid residues the same as that seen in PPA recognition, indicating that TtAAA-AT recognizes AAA and Glu with the very similar manner (Fig. 2).

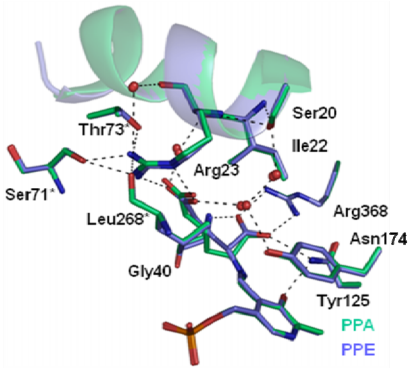

Fig. 2 Active site structures of TtAAA-AT in complex with PPE (purple) and PPA (green)

References

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