Biological Science

NW12, 5A, 6A /2007G530

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

Takeo TOMITA[1], Takuya OKADA[1], Tomoharu MIYAGAWA[1], Takuya OUCHI[1], Makoto NISHIYAMA*[1]
[1]The Univ. of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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 analysis 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. MAD data set of SeMet substituted TtHCS were collected using beamline NW-12. 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 to synthesize homocitrate, which is the first step of lysine biosynthetic pathway through AAA. We determined the first crystal structures of HCS complexed with Cu^{2+}/α-KG, Cu^{2+}/HC and Mg^{2+}/α-KG at 1.80, 1.96, and 2.15 Å resolution, respectively. HCS consists of $(\beta/\alpha)_8$ TIM barrel domain (Met1-Ala245) and C-terminal small domain I (Pro246-Ala320) (Fig. 1A). In the HC complex, the C1-carboxyl group of HC, which is derived from acetyl-CoA, is hydrogen-bonded with His292* from another subunit, indicating direct involvement of this residue in catalytic mechanism of HCS (Fig. 1B). The C5-carboxyl group of HC forms electrostatic interaction with Arg133, which clearly elucidated the substrate recognition mechanism of HCS (Fig. 1B).

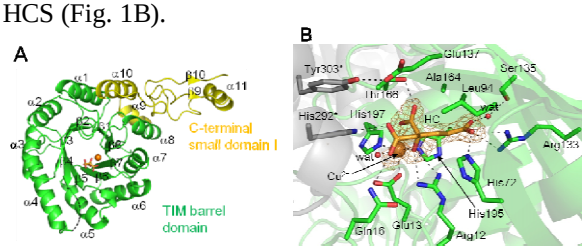

Fig.1 Crystal structure of TtHCS. A, Monomer structure, B, Active site structure in TtHCS/Cu^{2+}/HC complex.

TtAAA-AT[2]

TtAAA-AT, a homolog of mammalian kynurenine aminotransferase II (Kat II), transfers an amino group to 2-oxoadipate to yield AAA in lysine biosynthesis in *T. thermophilus*. We determined crystal structures of TtAAA-AT in four forms: PLP complex, PLP/Leu complex, PPL complex, and PPA complex at 2.67, 2.26, 1.75, and 1.67 Å resolution, respectively. The PLP complex is an open state, whereas the PLP/Leu, PPL, and PPA complexes are in closed states with maximal displacement over 7 Å of α2 helix and β1 strand in the small domain to cover the active site, indicating that TtAAA-AT takes multiple conformations to recognize various substrates (Fig. 2A-D).

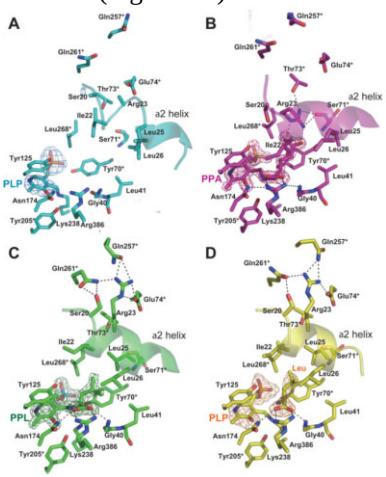

Fig. 2 Active site structures of TtAAA-AT in complex with PLP (A), PPA (B), PPL (C), and PLP/Leu (D).

References

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2) T, Tomita *et al. Proteins* (2009); **75**:248-359.

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