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Crystallographic analyses of enzymes involved in lysine biosynthetic pathway of *Thermus thermophilus*

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Introduction

Lysine is the essential amino acid for animals. Plants biosynthesize lysine via DAP pathway, while fungi or yeast biosynthesize lysine from AAA. On the other hand, we found that *Thermus thermophilus* synthesizes lysine via AAA pathway, although it is a bacterium. The enzymes involved in the former half of this pathway have similarity to those involved in leucine biosynthetic pathway or TCA cycle. The enzymes involved in latter half contain enzymes similar to those involved in arginine biosynthetic pathway. To investigate the mechanism of catalytic mechanism and substrate recognition in the enzymes of this unique lysine biosynthetic pathway, we preceed the crystallographic analyses of the enzymes involved in this pathway.

Materials and Methods

Preparation of crystals

HCS was expressed as hexahistidyl-tag fusion manner. The heat treatment, Ni-NTA affinity chromatography and following gel filtration chromatography were performed to purify. The crystal was obtained by vapor diffusion method with a reservoir solution which contains 2.0 M ammonium sulfate, at 20 °C in 2-3days.

AAA-AT was purified by the method as described previously (1). The synthesized *N*-phophopyridoxyl-leucine (PPL) and *N*-phophopyridoxyl-α-aminoadipate (PPA) were added in the protein solution to obtain those complexes. The crystal were obtained by vapor diffusion method . The reservoir solution contains 17.5-18.0 % (w/v) polyethylene glycol (PEG) 4000, 100 mM tri-sodium citrate (pH6.1-6.6) and 200 mM ammonium acetate for the PLP form, 8-13 % (w/v) PEG 6000 and 100 mM Tris-HCl (pH7.0) and 200 mM lithium sulfate for PPL form, and 18 % (w/v) PEG 3350 and 200 mM potassium fluoride for PPA form.

Results and Discussion

HCS/HCA complex

The structure of HCS was solved by multiple anomalous dispersion (MAD) method using the MAD diffraction data sets of 2.15 Å-resoluiton. The native data of 1.8 Å resolution was used for molecular replacement. 1 molecule of HCS exists in the asymmetric unit. Biological unit is dimer of symmetrical pair from distinct asymmetric units, which is corresponding to the previous observation in gel filtration and large buried surface area between two molecules. The monomer of HCS formed N-terminal $(\beta/\alpha)_8$ barrel and C-treminal small domain. In the active center, a metal ion, presumably Magnesium ion in crystallization mixture, and a HCS reaction product, homocitrate (HCA) were found. HCA was bound to HCS with specific hydrogen bonds with His72 and Thr166, and His195 and electrostatic interactions with Arg12 and Arg133. The attached acyl group by the reaction formed hydrogen bond with His292, which strongly indicates this residue is responsible for the proposed acid-base catalytic mechanism.

The structures of AAA-AT, AAA-AT/PPL and AAA-AT/PPA

The structures of AAA-AT, AAA-AT/PPL and AAA-AT/PPA were determined at 2.67, 1.75, and 1.67 Å resolutions, respectively. The structure of TtAAA-AT is an open form, while TtAAA-AT and TtAAA-AT are closed forms. The α2 helix is moved maximul over 7 Å to close to the active center and recognition of the substrates analog PPL and PPA. To recognize side chains of leucine (hydrophobic) and AAA(acidic), Arg23 has dual function; in that Arg23 (i) forms electrostatic interactions with Glu74 and hydrogen bond with Gln257 to stabilize the position of α1 helix for Ile22, Leu25, and Leu26 to form hydrophobic interaction with leucine moiety (ii) and forms electrostatic interaction with the γ-carboxyl group of AAA (Fig. 1).

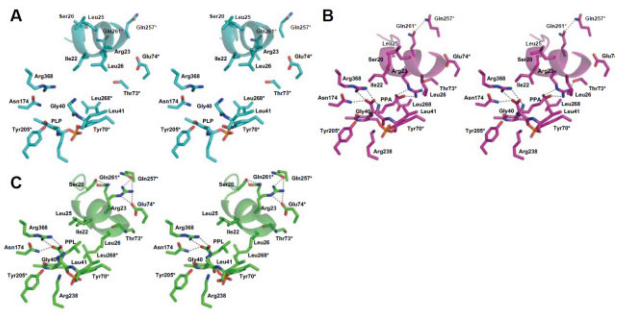

Fig. 1 The active centers of (A) PLP form, (B) PPA form, and (C) PPL form.

Reference

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