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Crystal structures of enzymes and transporters involved in amino acid metabolism

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Introduction

Lysine is produced industrially by *Corynebacterium glutamicum* mutants. *C. glutamicum* mutants with (*S*)-2-aminoethyl-L-cysteine (AEC) resistance were isolated, and their derivatives are used for industrial production of lysine.

Our recent crystallographic studies have proven that most of the mutations conferring AEC resistance are located at the regulatory domain of aspartate kinase (AK) from *C. glutamicum*. Although lysine is synthesized through diaminopimelate (DAP) pathway in most bacteria, it is synthesized through α-aminoadipate pathway in *Thermus thermophilus*. Lysine biosynthesis is regulated by two different stages in *T. thermophilus*: 1) feedback inhibition of homocitrate synthase (HCS), 2) transcriptional repression of genes involved in the biosynthesis. Interestingly, the growth of *T. thermophilus* was inhibited at 50 μM AEC, which contrasts with the cases for other bacteria, such as *E. coli* and *C. glutamicum*, which grow even in the presence of 500 μM AEC. This result indicates that *T. thermophilus* has hyper-sensitivity to AEC. Our previous study revealed that AEC inhibits HCS of *T. thermophilus* at μM levels. This suggested that the growth inhibition by AEC of *T. thermophilus* might be due to the inhibition of HCS. However, growth of a *T. thermophilus* mutant possessing HCS with H72L replacement, which gave complete AEC resistance to HCS, was also inhibited by AEC with sensitivity the same as that of wild-type strain. This result suggests that AEC acts on a target other than HCS to inhibit the growth of *T. thermophilus*. Analysis of the mutant strain with AEC resistance followed by its isolation revealed that two amino acids transporters were necessary for AEC resistance of the mutant. To clarify the mechanism of substrate recognition of the transporter, we performed crystallographic analysis of periplasmic substate-binding protein (PSBP) (TTC0807) complexed with AEC, Lys, Orn, and Arg.

Materials and Methods

Preparation of crystals and structure determination – Crystallization of TTC0807/AEC, TTC0807/Lys, TTC0807/Orn, TTC0807/Arg were performed by the hanging drop vapor diffusion method. The reserviour solutions for obtaining each complex are shown below.

TTC0807/AEC complex: 0.2 M sodium sulfate, 20% PEG 4000, 10 mM AEC. TTC0807/Lys complex: 0.2 M ammonium sulfate, 20% PEG 4000, 10 mM lysine. TTC0807/Orn complex: 0.2 M sodium sulfate, 20% PEG 4000, 10 mM ornithine. TTC0807/Arg complex: 0.2 M ammonium sulfate, 20% PEG 4000, 10 mM arginine

The structure of TTC0807/AEC complex was determined by molecular replacement method using the structure of StLAO-BP (PDB code, 1LST).

Results and Discussion

Overall structure of TTC0807 – TTC0807 was consisted from domain I and domain II. Structrure of TTC0807 was similar with that of StLAO-BP, while the hinge between the domains were replaced to a β-sheet β8-β12 (Fig. 1).

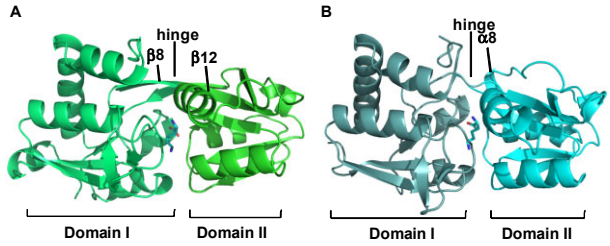

Fig. 1 Crystal structure of TTC0807/AEC complex (A) and StLAO-BP (B).

Substrate binding pocket of TTC0807 – The bound substrates were recognized by similar manner, while conformations of Glu19 in those complexes were altered to accommodate the different substrates.

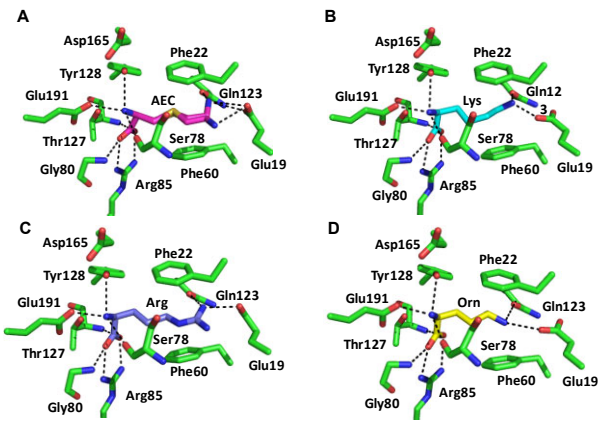

Fig. 2 Substrate binding pocket of TTC0807 complexed with AEC (A), lysine (B), arginine (C), and ornithine (D).

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