NW12, NE3, 5A /2011G525

Crystal structures of the enzymes involved in novel lysine biosynthetic pathway using amino acid carrier protein

Takeo TOMITA[1], Ayako YOSHIDA[1], Kento TAKAHASHI[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 analyses of enzymes and proteins involved in lysine biosynthetic pathway. Here, we describe the recently determined crystal structures of TK0278 and Saci_0600.

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

TK0278

The genes coding the enzymes involved in lysine biosynthetic pathway *via* AAA are found in several bacteria and Archea. Hyperthermophilic archea, *T. kodakarensis* also has the gene cluster of AAA pathway. However, it has no genes encoding arginine biosynthetic enzymes which have similarity with AAA lysine biosynthetic enzymes. In the genome of *T. kodakarensis*, there is one copy (*TK0278*) of *lysX* homolog, encoding the first characteristic enzyme in the latter part from AAA of the pathway. We suppose that TK0278 can catalyzes two reactions involved in lysine and arginine (ornithine) biosynthesis, that is, TK0278 can ligate AAA and glutamate to C-terminal glutamate residue of LysW homolog (TK0279) in ATP-dependent manner. *In vitro* assay, we confirmed that TK0278 catalyzes the both reactions and are involved in both lysine and arginine biosynthesis. To elucidate structural basis of the bifunctionality of TK0278, we crystalized TK0278 with TK0279 (LysW), AMP-PNP, and AAA or glutamate. So far, we determined the crystal structure of TK0278 with AMP-PNP. It forms a tetramer (Fig. 1). The structure provides candidate of the residue involved in both AAA and glutamate bindings, which lies at the substrate-binding site.

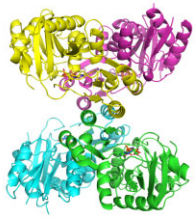

Fig. 1 Overall structure of TK0278

Saci_0600

The forth reaction of lysine biosynthesis in *Thermus thermophilus* is catalyzed by homoisocitrate dehydrogenase (HICDH). On the genome of *Sulfolobus acidocaldarius*, Saci_0600 is annotated as a homologous enzyme, isopropylmalate dehydrogenase (IPMDH) in leucine biosynthesis and Saci_2375 is annotated as isocitrate dehydrogenase (ICDH) in tricarboxylic acid cycle, while HICDH gene is not found. Saci_0600 possesses IPMDH activity. To elucidate the mechanism of substrate recognition, we determined the crystal structure of Saci_0600 complexed with 3-isopropylmalate (3-IPM) at 2.2 Å resolution. Saci_0600 formed tetrameric structure. Saci_0600 has compact loop structure in its active site while IPMDH from *Thiobacillus ferroxidans* has extended loop structure. Saci_0600 recognizes the γ-moiety of 3-IPM by hydrophobic region consist of Ala78, Ala79, Val82, and Val83 (Fig. 2).

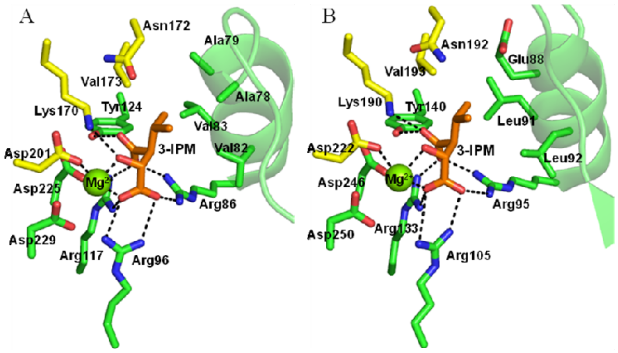

Fig. 2 Comparison of active sites between Saci_0600 complexed with 3-IPM/Mg^{2+} (A) and IPMDH from *Thiobacillus ferrooxidans* complexed with 3-IPM/Mg^{2+} (B)

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