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, and progress of data analysis of TK0283.

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 archaea. This biosynthetic system uses small acidic protein called LysW as a protecting-group of α-amino group and also as a carrier protein. Moreover, we recently found that hyperthermophilic archaea, *Sulfolobus* biosynthesizes not only lysine but also arginine with this system. Hyperthermophilic archea, *T. kodakarensis* also has the gene cluster of LysW-mediated pathway. 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 have already identified that TK0278 can catalyzes two reactions involved in lysine and arginine (ornithine) biosynthesis *in vitro*. To elucidate structural basis of the bifunctionality of TK0278, we crystallized TK0278. So far, we determined the crystal structure of TK0278 with AMP-PNP. From the structure, we could suggest some residues involved in substrate binding. Since some mutants of substrate-binding residues showed activities like other monofunctional LysX homologs, the residues recognizing two different substrates were identified. However, we want to know how TK0278 recognizes both AAA and glutamate more precisely, so now we are trying to obtain the new crystal of TK0278 with all substrates; LysW, ATP, and AAA or glutamate.

Saci_0600 and TK0283

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. 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 of the enzyme. We also tried structure analysis of TK0283 from *T. kodakarensis*. TK0283 is an enzyme annonated as as homoisocitrate dehydrogenase (HICDH) involved in lysine biosynthesis. TK0283 is thought to be a multi-fucntion enzyme with broad substrate specificity. We obtained crystals of TK0283 in presence of several substrates. We observed diffraction from several of the crystals. Improvement of resolution of diffraction is tried now.

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