AR-NW12, AR-NE3A, BL-5A/2013G618

Crystal structures of enzymes and transporters involved in amino acid metabolism

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Introduction

Ancestral enzymes are believed to have broad substrate specificity to maintain multiple physiological functions by one enzyme because genome size of ancestral organisms is predicted to be smaller than that of today's life such as eukaryotes and mesophilic prokaryotes. According to patchwork hypothesis proposed by Jensen, duplication of genes encoding such a multifunctional enzymes allows them to specialize their substrate specificity, as a result most enzymes found in today's life have strict substrate specificity with high catalytic efficiency. We recently found that evolutionary ancient hyperthermophilic archaea, *Sulfolobus* biosynthesizes both lysine and arginine by a single set of bifunctional enzymes and a common carrier protein called LysW. Our discovery indicated that validity of the patchwork hypothesis within evolution of amino acid biosynthetic enzymes, and thus further analysis of enzymes from hyperthermophilic archaea may provide clues to understand evolutionary process of ancestral enzymes as well as ancestral metabolic pathways.

Hyperthermophilic archaea, *Thermococcus kodakarensis* is known as one of the most ancient organisms. We have already identified that its putative lysine biosynthetic enzymes can catalyze two reactions involved in lysine and arginine (ornithine) biosynthesis *in vitro*. Moreover, enzymes involved in the first half of putative lysine biosynthetic pathway in *T. kodakarensis* seem to catalyze reactions involved in lysine biosynthesis as well as glutamate and leucine biosynthesis because in the genome of *T. kodakarensis*, there is no paralogous genes required for biosynthesis of latter two amino acid biosynthesis. To elucidate structural basis of multifunctional enzymes, we have determined crystal structures of TK0280 catalyzing β-decarboxylating dehydrogenation involved in lysine, glutamate and leucine biosyntheses in *T. kodakarensis*.

Materials and Methods

Purification of TK0280

TK0280-His_6 was overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as a host using 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) for induction. From cell lysate prepared by sonication, TK0280 was purified through heat treatment, Ni^{2+}-NTA affinity chromatography and Superdex 200 gel filtration chromatography.

Crystalization of TK0280

Screening of crystallization condition was initially performed with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method. The crystallization conditions were further optimized by changing pH and concentration of precipitant.

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

Purification and characterization of TK0280

Through heat treatment and successive column chromatographies, TK0280-His_6 was purified to homogeneity on SDS-PAGE analysis. Purified enzyme showed NAD^+ dependent β-decarboxylating dehydrogenation with homoisocitrate (HIC), isocitrate (IC) and 3-isopropylmalate (IPM) as substrates confirming that TK0280 can catalyze reactions involved in lysine, glutamate and leucine biosynthesis.

Overall structure of TK0280

As a result of crystal screening, we determined structure of TK0280 apo form and complex with HIC at 1.7 and, 2.6 Å resolution, respectively. Apo structure of TK0280 was tetramer same as reported in homoisocitrate dehydrogenase (HICDH) from *Thermus thermophilus* while structure of TK0280/HIC complex showed distinct oligomeric state. Domain closure induced by binding of HIC was observed in TK0280/HIC complex structure comparing to apo structure. However, improvement of resolution of diffraction is desirable for TK0280/HIC complex to determine detailed structure of C-terminal domain. We are also trying to crystallize TK0280/IC and TK0280/IPM complex to elucidate structural basis of broad substrate specificity of TK0280.

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