Structural Studies on Thermal Stabilization of Enzymes

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Introduction

Several experimental theories about protein thermostability have been proposed.We also have been tried to obtain new views about structural basis of thermostability with x-ray crystallography.

We chose two hyperthermophilic protein; Aspartate aminotransferase (PlAspAT) from *Phormidum lapideum* and isopropylmalate dehydrogenase (SulIPMDH) from *Sulfolobus* strain. Both strain lives in over 80°C and their enzymes have also high thermostability.

PlAspAT

Phormidum lapideum is a thermophillic cyanobacterium living in hot spring. PlAspAT catalyzes reversible transfer from L-aspartate to α-ketoglutarate, with pyridoxal phosphate as a coenzyme. Its optimal temparature is about 80°C.

SulIPMDH

IPMDH is an enzyme in the leucine biosynthetic pathway. It catalyzes the dehydrogenation and decarboxylation in the conversion of 3-isopropylmalate to 2-oxoisocaproate, with the reduction of NAD^+. IPMDH from *Sulfolobus* sp. strain 7 has a Tm value of 97°C, while E.coli IPMDH has a Tm of 64°C.

Experimental and Results

PlAspAT

The crystal was soaked in the reservoir solution containing 20% glycerol as cryoprotectant for a few minutes. After soaking, the crystal was mounted on the loop and quickly transferred to 100K cold stream.

Diffraction data was collected at the BL41XU station of the SPring-8. The X-ray wavelength was set to 0.708Å. Fifty-five plates of its oscillation image with 1.5° oscillation angle were taken, but integration and scaling have not done yet.

SulIPMDH

As cryoprotectant, 20% glycerol was added into reservoir solution. Soaking and mouning were done with the same way as PlAspAT.

Diffraction data was collected at the BL41XU station of the SPring-8. The X-ray wavelength was set to 0.708Å. The intensities were integrated with the program DENZO and scaled with the program SCALEPACK. About fifteen thousand independent reflections were observed within 100-2.8Å resolution. The R-merge and the completeness of this data set were 15% and 70%, respectively. The observed amplitudes were quite different from the previous datasets in spite of the same cell dimensions.

With these reflections, Patterson map and self-rotation function were calculated. A prominent peaks was found on the Harker secion along the Z, although no big peaks were on (u 0 v) and (0 v w) planes. It might be caused by non-isomorphism or twinning of the crystal.