Structure Analysis of Enzymes from a Hyperthermophilic Archaeon

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Introduction

Pyrococcus horikoshii is a hyperthermophilic archaeon that grows at a temperature near the boiling point of water. Its proteins are highly thermostable and have been expected to be useful for the industrial application. We have been investigating the structure and function of enzymes from the archaeon, of which optimal temperature is in the range from 90ºC to 100ºC. The comparison of their structure and function with those from non-thermophilic organisms will shed light on the elucidation of the mechanism of thermostability of proteins.

Experimental

X-ray measurements in PF have been carried out at BL-6A beam line. The monochromated X-ray with the wavelength 1.0 Å was used after passing through the aperture collimator of 0.1 mm diameter. Low temperature experiments were carried out at near 100K with a crystal mounted in a cryo-loop. Oscillation photographs were taken by using the ADSC Quantum 4R CCD detector. The data were processed by DPS/MOSFILM and data-reduction programs incorporated in the CCP4 package.

Results

Aromatic Amiotransferase

Previously we have reported the crystal structure of aromatic amino acid aminotransferase from *Pyrococcus horikoshii* [1]. The structure was solved for the X-ray data measured by using rotating anode generator. However, twelve amino acid residues in the N-terminal region could not be modeled because of the poor electron density. To obtain the complete model of the structure, we tried to collect the high-resolution data under cryo-cooling. Several crystals were subjected for data collection at 100K. But the diffraction quality of the crystal was poor at high resolution because of the increase of mosaicity. We did not use the cryo-solvent because the protein was crystallized with PEG6000 and 1,6-hexane-diol as precipitants and the solution was clear at 100K. The result indicated the necessity to try some cryo-solvents to improve the diffraction quality of the crystal.

Flap Endonuclease

This enzyme cuts off the flap region of double-helical DNA strands in the process of DNA recombination and repair. Two crystal structures of thermophilic archaea have been reported but the mechanism of DNA binding and enzyme action has been controversial because of the lack of structural information of protein-DNA interaction. We have expressed the enzyme from *Pyrococcus horikoshii* and the wild-type and several mutant enzymes have been subjected for crystallographic study. Two crystals have been obtained for the Arg42Glu mutant protein. One belongs to a monoclinic space group $P2_1$ while the space group of the other crystal was a trigonal $P3_1$. The former crystal diffracted to the resolution of 2.5 Å but the diffraction was limited to ca. 4 Å resolution in the direction of a* axis. The other crystal diffracted to ca. 3 Å resolution. The structure was solved for the former crystal by the molecular replacement but the refinement had severe difficulty in the model building of some loop regions because of the poor resolution in the direction perpendicular to the ac plane in the electron-density map. In the crystal, the asymmetric unit contains two molecules that are related by a local twofold axis. The molecular replacement study has revealed the same dimer structure in the other crystal. The structure refinement is in progress.

Tk-REC

The REC protein plays an important role in the recombination and repair of DNA. Previously we have reported the crystallization of the protein from *Thermococcus kodakaraensis*, a hyperthermophilic archaeon [2]. The protein cryatallized in the two forms with the space group $P2_12_12_1$ with PEG1000 and PEG550 monomethylether. However, the asymmetric unit of these crystal contained more than 20 molecules and not suitable for the structure determination. Several trials of crystallization using PEG6000 as a precipitant gave another crystal with the same space group. The asymmetric unit of this crystal contained two molecules and the X-ray diffraction was observed beyond 3 Å resolution for a crystal with the dimension less than 0.1mm. We have succeeded to collected a set of native data at 3 Å resolution but the result indicates we need larger crystals for the structure determination by the MIR method.

References

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