

Crystallographic study of molecular mechanism of the thermostability of DNA binding proteins

Hideaki Koike(4278)

CREST Centre of Structural Biology, AIST-NIBHT

The 3D structure of the TATA-box binding protein (TBP) from a thermophilic archaeobacterium, *Sulfolobus acidocaldarius*, has been determined in the resolution of 2.0 Å with the R-factor being 20.4%.

Crystals of the TBP were obtained by the vapour diffusion method using lithium sulfate and ammonium sulfate as precipitants. Using x-ray source of synchrotron radiation at the facility of SPring8 (beam line 24A), x-ray was diffracted up to 2.0 Å, while up to 3.0 Å using laboratory x-ray source. The higher resolution data made it possible to determine a 3D model of the protein of good quality and to analyse structural element contributing to thermostability of protein.

The crystal was identified to be of the orthorhombic space group, P21212, and thus is different from any other reported crystals of TBPs. Two independent molecules were identified positioning in the asymmetric unit of 64.0x81.2x88.1 Å. A 3D model was constructed by the molecular replacement method. In the final structure the overall combination of the secondary structures was found essentially kept the same, but differences in position and conformation were observed in the residues in distal region from the centre of the molecules and loop connecting the region and the central β -strand. Short α -helices are distorted.

On the basis of the determined TBP structure

and known TBP structures of other species, and of the amino acid sequences of TBPs of archaebacterial species that possess different optimal growth temperature values, the domainal thermostability and the inter-domainal thermal flexibility are being studied. The TBP of a hyper-thermophilic archaeobacterium, *Pyrococcus* sp. OT3 (Opt. T., close to 100°C) is, in general, more stable than TBPs of two thermophilic archaebacterial species, *Sulfolobus acidocaldarius* (Opt. T., 75°C), and *Thermoplasma volcanium* (Opt. T., 60°C). The TBP from *Sulfolobus* showed higher thermal stability upon decreasing the KCl concentration, while the other two TBPs, in contrast, upon increasing the KCl concentration. Specific amino acid residues and more global factors that determine the thermostability and its salt dependence are being investigated. An amino acid position that is possibly important for the thermal flexibility has been identified on the inter-domainal surface, which is occupied by a large aromatic residue, Phe, in the TBP from *Pyrococcus*, and a small residue, Ala, in that from *Thermoplasma* and *Sulfolobus*, while the smallest residue, Gly, in those of the eukaryotic origin.