Temperature Dependence of the Enzyme-Substrate Recognition Mechanism

Hideaki URA[1], Kazuaki HARATA[2], Ikuo MATSUI[2], Seiki KURAMITSU[*1]
[1]Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan
[2]National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki 305-8566, Japan

Introduction

The thermostability of thermophilic enzymes has been extensively studied, and it has been suggested that the forces contributing to their thermostability are increases in electrostatic interactions, hydrogen bonding, and packing. Usually there are no critical residues for increasing the thermal stability of a protein, but rather cumulative small contributions of many residues. However, the temperature dependence of the substrate recognition mechanism remains to be elucidated.

Methods

The crystallization of the aromatic amino acid aminotransferase from *Pyrococcus horikoshii* (PhAT) was performed by the hanging-drop vapor diffusion method at 20°C using 3 M 1,6-hexane-di-ol, 100 mM HEPES (pH 7.5), and 10 mM $MgCl_2$ as the precipitant solutions for the unliganded (Native) and *N*-5'-phosphopyridoxyl-L-glutamate (Complex) forms. X-ray diffraction data for all forms of PhAT were collected on the BL6A station at the Photon Factory, KEK (Tsukuba), using an X-ray beam of 1.0 Å wavelength and an ADSC Quantum 4R CCD detector. Images were integrated with the MOSFLM package, and subsequent data processing and refinement were performed with the CCP4 package, XPLOR version 3.851 and program O. The details of the data collection and processing statistics are given in Table 1.

Table 1: Data collection and refinement statistics

	Native	Complex
Source (Å)	1.0	1.0
Temperature (K)	100	100
Diffraction data		
Space group	$P2_12_12_1$	$P2_12_12_1$
Lattice constants		
a (Å)	59.67	59.33
b (Å)	122.28	121.90
c (Å)	127.17	127.01
Resolution (Å)	1.8	1.8
Observations	272,833	309,808
Unique reflections	83,030	84,494
Completeness (%)	96.0	98.6
R_{merge} (%)	8.6	5.3
Refinement		
Resolution limits (Å)	8.0–1.8	8.0–1.8
R_{factor} (%)	21.0	19.8
R_{free} (%)	24.2	23.0

Results and Discussion

Aspartate aminotransferase from the mesophile *Escherichia coli* (EcAT) consists of two domains, which close upon substrate binding [1, 2]. Recently, we were able to estimate the free energy required for domain movement by means of kinetic studies in a series of aliphatic substrates and corresponding crystallographic studies [3]. Upon binding of the acidic substrate, the side chain of Arg292* largely moves into the active site to create bifurcated hydrogen bonds and an electrostatic interaction with the distal carboxylate (ω-carboxylate) group of the bound acidic substrate.

The aspartate aminotransferase from an extreme thermophile, *Thermus thermophilus* HB8 (TtAT), is a homologue of EcAT [4]. No large domain movement is observed upon binding of a substrate to TtAT. The single N-terminal α-helix consisting of Lys13 to Val30 approaches the bound substrate and closes the active site. Ser15 and Thr17 in the N-terminal region of the α-helix interact with one of the carboxylate oxygen atoms of the bound acidic substrate, and Lys109 interacts with another oxygen atom without movement of its side chain.

Upon binding of an acidic substrate to hyperthermophilic PhAT [5], little domain movement was observed, except for a small movement of the α-helix from Glu16 to Ala25. The ω-carboxyl group of the acidic substrate was recognized by Tyr70* without its side-chain movement, but not by positively charged Arg or Lys.

By comparing enzymes from a hyperthermophile, an extreme thermophile and a mesophile, we have found that they appear to obey a general rule: "the more thermophilic the enzyme is, the smaller the domain movement upon binding to a substrate is." It will be of considerable interest to determine whether this rule is applicable to the enzymes of psychrophiles [6].

References

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*kuramitu@bio.sci.osaka-u.ac.jp