Mechanism for thermo-stabilization of enzymes from view of the three dimensional structure

Yuji YOSHIIKE (3084), **Hideaki MORIYAMA** (1251), **Kazuhiko KAKOI**§ (3353), **Ryuji FUJIHARA**§ (3473), **Katsuhiko OKAMOTO**§ (3503), **Kensaku HAMADA**§ (1241), and **Nobuo TANAKA*** (3080)

Faculty of Biotechnology and Bioscience, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, 226-8501 Japan
§Interdisciplinary Faculty of Science and Engineering, Shimane University, 1060 Nishi kawatsu-cho, Matsue, Shimane 690-8504 Japan

The physiological properties of organisms have been determined by consequence of serial events in physical, chemical, biochemical and biological, under certain environment. At a shape of projection , the evolution can be interpret as the adaptation of middle temperature from the hot water world. The comparisons of protein stability of present organisms that live extreme and mild environment give the physicochemical mechanism. The authors have established the structure analysis of proteins from thermophiles [1].

Aspartate aminotransferase (AspAT) from *P. lapideum* (an blue-green alga; origin of Matsue, Japan) is a homo dimmer enzyme with 338 amino acids in a subunit and it has stable activity at 80 °C.

While the structures of AspATs belong to different class including pig [2], chicken [3] and *Escherichia coli* [4] were known, that from blue-green alga, alga [5] and *Thermus aquaticus* [6] were unknown. The structure analysis of AspAT from *P. lapideum* elucidate the mechanism of heat stability.

The enzyme was amplified in *E. coli*, purified, and crystallized by the hanging drop vapor diffusion method using ammonium sulfate. At the laboratory level CuKα X-ray, diffraction could not record beyond 6 Å from the crystal. Then the crystals, 20x20x250 μm were mounted in a glass capillary and diffraction SR experiments were done at BL41XU. The crystal gave reflections maximum 2.0 Å Bragg spacing using wave length at 1.00 Å with oscillation angle ($\Delta\omega$) 0.5° at 1.0°/S with 12 times of oscillation. For the data collection, the oscillation photographs were taken with $\Delta\omega$ 1.5° and 24 - 30 swings. During the experiment, ring current was between 14.4 and 12.4 mA. Although the crystal diffracts well in the SR experiment, the damage of crystal occurs within 78S of irradiation at the room temperature even it is a heat stable protein. The suppression of the potential damages must employ to the experiment by mean of cryo-crystallography to achieve the data collection. The research will continue on this way.

Orotate phosphoribosyltransferase (OPRT) from *Thermus thermophilus* HB27 plays an essential role in the *de novo* biosynthesis of pyrimidine nucleotide. Its optimum temperature is 75 °C, and it is stable to 85 °C [7]. Although the crystallization conditions and 3D structures of mesophilic OPRTs from *Salmonella typhimurium* [8] and *E. coli* [9] have been determined, the thermophilic structures have not. To clearfy the structural background of the thermostability in OPRT from *Thermus*, knowledge of the 3D structure is indispensable, due to the lack of primary-sequence homology among these OPRTs.

OPRT was purified and crystallized by means of the hanging drop vapor diffusion method with macro seeding technique. X-ray diffraction experiments were performed using labo mashine resulted the crystal belongs to the monoclinic space group $P2_1$ with the unit cell dimensions of a = 44.4, Å, b = 59.6 Å, c = 67.7 Å,, β = 98.3°. Diffraction data were collected for up to 2.1 Å resolution. To obtain the further reflection data, SR experiment was done with the same manner as AspAT. The crystal diffract up 1.8 Å resolution and 10 images were processable out of 24 images.

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