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Medium temperature, 310 K, provides single crystals of orotate phosphoribosyltransferase from *Thermus thermophilus*

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Crystallization of orotate phosphoribosyltransferase from a thermophilic organism, *Thermus thermophilus*, was achieved using the hanging-drop vapour-diffusion method coupled with a macroseeding starter. Small needle-like microcrystals were grown in a fresh protein solution in the presence of 2-methyl-2,4-pentanediol at 298 K or below. Although these normal temperature conditions caused stacking crystallization, an increase of temperature to 310 K permitted crystal growth. This was because of increased enzyme solubility at the higher temperature. The crystal was found to belong to the monoclinic space group $P2_1$ with unit-cell parameters a = 44.4, b = 59.6, c = 67.8 Å and β = 98.3°.

1. Introduction

Protein concentration, ionic strength, pH, temperature and sometimes gravity (Chayen *et al.*, 1996) are important determinants in the process of crystallization. The manner of applying these variables to control the power of van der Waals dispersion during crystallization depends on the nature of the target protein. Among these variables, temperature is generally set at or below room temperature to maintain the quality of the protein. In some cases, however, high temperature (McPherson, 1985) and high pressure (Gross & Jaenicke, 1994; Visuri *et al.*, 1990) increase the protein solubility, whilst keeping the protein folding intact. Control of temperature then provides a slow phase transition from solution to solid crystal. Our results showed the occurrence of these phenomena in the crystallization of this enzyme as well as ferredoxin (Fujii *et al.*, 1997).

Orotate phosphoribosyltransferase (OPRTase, E.C. 2.4.2.10) from *T. thermophilus* HB27 (Bunnak *et al.*, 1995; Yamagishi *et al.*, 1996) plays an essential role in the *de novo* biosynthesis of pyrimidine nucleotides. It catalyzes the formation of the nucleotide orotidine monophosphate from the ribose donor α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) and the nitrogenous base orotic acid. OPRTase has a subunit molecular mass of 20130 and is a dimeric enzyme. Its optimum temperature is 348 K and it is stable to 358 K. Although the crystallization conditions and three-dimensional structures of mesophilic OPRTases from *Salmonella typhimurium* (Wallon *et al.*, 1997) and *Escherichia coli* (Henriksen *et al.*, 1996) have been determined, the thermophilic structures have not. To clarify the structural basis of the thermostability of OPRTase from *T. thermophilus*, knowledge of its three-dimensional structure is indispensable as there is a lack of primary-sequence homology among these OPRTases. The present enzyme isolated from *T. thermophilus* has an amino-acid sequence that is 28 residues shorter than those of *E. coli* (Poulsen *et al.*, 1984) or *S. typhimurium* (Scapin *et al.*, 1993). The sequence identity of these two enzymes is 97%, while the identity of these two OPRTases and that of *T. thermophilus* is 26%, excluding deletions. The amino-acid compositions are also different, suggesting a hydrophobic character for *T. thermophilus* OPRTase.

In this report we describe the crystallization and preliminary X-ray studies of the enzyme OPRTase from *T. thermophilus*.

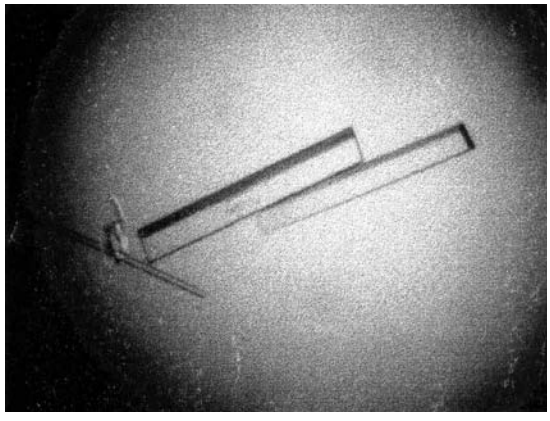

Figure 1
A microphotograph of OPRTase crystals.

2. Preparation, crystallization and data collection

OPRTase was purified by affinity chromatography with Blue Sepharose CL-6B (Pharmacia, Sweden), in addition to the procedure reported by Bunnak *et al.* (1995). Initial attempts at crystallization of OPRTase by the micro sitting-drop vapour-diffusion method were performed using crystal screen kits (Hampton Research, USA) with different reservoir sizes for the different dilutions. A crystal shower was observed under conditions of 18% polyethylene glycol 400, 0.1 *M* sodium HEPES pH 7.5 and 0.1 *M* $MgCl_2$. Other attempts were made using a simple batch method with ammonium sulfate as a precipitant and varying the temperature between 277, 288, 293, 298 and 310 K. Micro-needle crystals of size 0.25 × 0.025 × 0.025 mm were obtained

from a batch containing 40 mg ml^{-1} protein and 30% saturated ammonium sulfate in 20 m*M* Tris–HCl pH 7.5 after one week at 298 K. To obtain larger crystals, a hanging-drop vapour-diffusion method and macro-seeding technique were applied using the microcrystals as seed crystals. These crystals grew to a typical size of 1.0 × 0.4 × 0.2 mm, but consisted of a stacked assembly of thin plates. In an unsuccessful attempt to avoid the stacking, 0.1% 2-methyl-2,4-pentanediol was added to the crystal-growth solution. To slow the crystal growth, the protein solubility was increased by raising the temperature to 310 K, which resulted in single crystals of a typical size of 0.3 × 0.1 × 0.1 mm (Fig. 1). These crystals were grown in a drop containing 3 ml of 30 mg ml^{-1} protein, 2 ml of reservoir solution and 1 ml solution of seed crystals mixed and equilibrated against 500 ml of reservoir solution containing 23–24% saturated ammonium sulfate and 0.1% 2-methyl-2,4-pentanediol in 20 m*M* Tris–HCl pH 7.5. This setup was maintained at 310 K for 1–2 weeks.

X-ray diffraction experiments were performed using a Rigaku R-AXIS IIc imaging-plate diffractometer with Ni-filtered Cu $K\alpha$ radiation. A series of still and oscillation photographs were taken in oscillation mode. The resulting images indicated that the crystal belongs to the monoclinic space group $P2$ with unit-cell parameters a = 44.4, b = 59.6, c = 67.7 Å, β = 98.3°. The density measurements showed two subunits in each asymmetric unit with an average V_m value of 2.4 Å^3 Da^{-1}. The crystal diffracted to 2 Å resolution with an estimated mosaic spread of 0.3°. Diffraction data were collected to 2.1 Å resolution with overall and outer-shell completenesses of 89 and 69%, respectively. The reliability factor among symmetry-related reflections was 7%. Structure analysis is now under way using the heavy-atom isomorphous replacement method.

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