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Crystallization and preliminary X-ray studies on the hyperstable 3-isopropylmalate dehydrogenase from the thermoacidophilic archaeon *Sulfolobus* sp. strain 7

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Abstract

3-Isopropylmalate dehydrogenase from the thermoacidophilic archaeon, *Sulfolobus* sp. strain 7, has been crystallized by the vapor-diffusion method. The crystals were grown from a solution containing ammonium sulfate, 2-methyl-2,4-pentanediol and magnesium chloride. The crystallization requires 2-methyl-2,4-pentanediol to avoid twinning of the crystals. The crystal belongs to the orthorhombic system with the space group $P222_1$ and unit-cell dimensions $a = 67.9$, $b = 93.3$ and $c = 134.1$ Å.

1. Introduction

Archaebacteria including *Thermoplasma*, *Halobacterium*, *Methanopyrus* and *Sulfolobus* live in a severe environment in which the survival of eubacteria and eukaryotic cells is difficult. To survive such high temperatures, 353 K or higher, and extreme high- and low-pH conditions each archaebacterium has a unique metabolic pathway that permits these conditions. Many studies have been carried out to understand why proteins produced by archaebacteria offer this resistance. The structural and functional comparative studies on 3-isopropylmalate dehydrogenase (E.C. 1.1.1.85, IPMDH) from thermophiles, *Thermus thermophilus* (Imada *et al.*, 1991), mesophiles and chimeric enzymes have provided many insights (Miyazaki *et al.*, 1994; Onodera *et al.*, 1994; Kadono *et al.*, 1995). In the *Thermus* IPMDH, one important key for thermostability is the lowering of unfavorable structural constraints by keeping the flexibility of the molecule. Also systematic studies by means of site-directed mutagenesis and structure analysis of lysozyme (Gray *et al.*, 1996; Luque *et al.*, 1996) and ribonuclease (Gilis & Rooman, 1996; Kanaya *et al.*, 1996) have revealed that increases in rigidity as well as increases in hydrophobicity at the mutation site contribute to stabilizing the molecule against heat by altering the enthalpy level. However, structural and biochemical studies on the natural and the more thermostable enzyme have a large potential to provide further information.

Sulfolobus sp. strain 7 has been isolated as a new and unique member of an acido-thermophilic archaeon that live in an environment of pH 2–3 and 353–363 K, and have been found to have many interesting biological properties (Iwasaki *et al.*, 1995). Recently, the gene for an IPMDH from *Sulfolobus* sp. strain 7 was cloned and sequenced (Yoda *et al.*, 1995; Suzuki *et al.*, 1998). The behavior of the enzyme indicates that it has hyperstability against the heat. The temperatures of 50% inactivation, $T_{1/2}$, of *Sulfolobus* and *Thermus* IPMDH are 363 and 353 K, respectively. The enzyme shows stronger thermostability than any IPMDH reported so far and has a molecular weight of 37 kDa with 337 amino acids in a monomer subunit. Its mass was estimated to be 148 kDa by gel filtration. While homodimeric structures are known for other IPMDH's, only *Sulfolobus* IPMDH has a homo-tetrameric quaternary structure. To elucidate the structure and thermostability relationships, we have initiated crystallographic studies on the enzyme.

2. Crystallization, data collection and discussion

To determine the optimal conditions for crystallization of the present enzyme the hanging-drop method was employed. As the reservoir solution we used different crystal-screen-kit dilutions (Jancarik & Kim, 1991) and ammonium sulfate with different pHs, as reported for other IPMDH crystallizations (Imada *et al.*, 1991; Onodera *et al.*, 1994). The best conditions found were as follows. The hanging drops were prepared by the mixing of 4 µl of 28 mg ml^{-1} enzyme in 100 m*M* HEPES–NaOH buffer, pH 8.0, and 4 µl of reservoir solution containing 1.7 *M* ammonium sulfate, 5 m*M* $MgCl_2$ and 2.0–2.3% 2-methyl-2,4-pentanediol (MPD) in the same HEPES buffer. The drops were equilibrated against the reservoir solution. The addition of MPD contributes to the formation of single crystals, although the optimal concentration of MPD varied between different preparation batches. The presence of magnesium enhances crystallization. This may be due to the shrinkage effect of magnesium, as was found in *Thermus* IPMDH (Hurley & Dean, 1994; Kadono *et al.*, 1995).

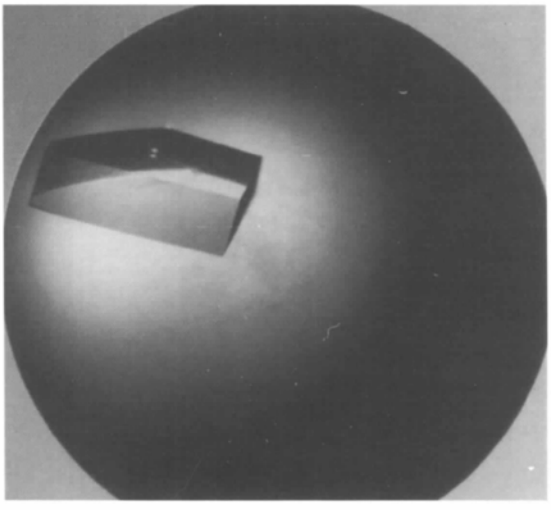
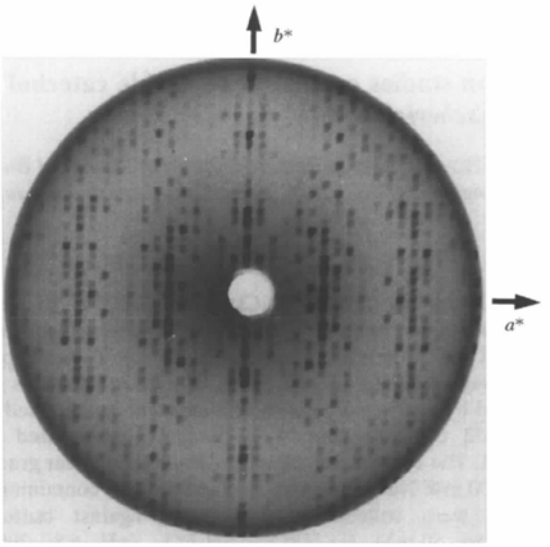
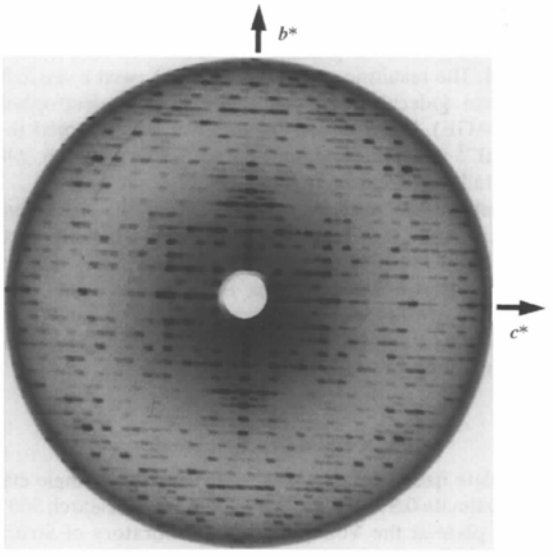


Fig. 1. Micrograph of a *Sulfolobus* IPMDH crystal. The approximate dimensions are 1.1 × 0.6 × 0.4 mm. The MPD concentration was 2.25%.



(*a*)



(*b*)

Fig. 2. Precession photographs of *Sulfolobus* IPMDH crystal. Planes are (*hk*0) (*a*) and (0*kl*) (*b*). The diameters of the original photographs are 50 mm.

A typical crystal is shown in Fig. 1. A single crystal was mounted in a thin-walled glass capillary and sealed together with mother liquor. To confirm the space group of the present crystal, precession photographs were taken in two different orientations using Ni-filtered Cu $K\alpha$ radiation with $\mu = 12°$ and a crystal-to-film distance of 60 mm. The photographs showed *mm* symmetry (Fig. 2) and there were systematic extinctions according to the condition $l = 2n$. V_m is calculated to be 3.0 Å^3 Da^{-1}, with respect the eight subunits in the unit cell. Thus, the space group was determined to be $P222_1$ with unit-cell dimensions $a = 67.9$, $b = 93.3$ and $c = 134.1$ Å. The precession photographs showed a second systematic increase of intensities of the reflections along the a^* axis with a spacing of 11 Å. This may be the result of periodic and helical packing of the IPMDH molecules in the crystal. X-ray diffraction photographs were taken in still and oscillation modes using a Rigaku R-AXIS IIc diffractometer. The crystal diffracts up to 2.5 Å resolution. A data set comprising 61 oscillation photographs with an oscillation range of 1.5° was collected to 2.8 Å resolution. The data consist of a total of 68 657 observations of 19 762 independent reflections for which the $R_{\rm merge}$ and completeness were 5 and 95%, respectively. The structure determination on the present crystal was now under way using the heavy-atom isomorphous replacement method.

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