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Crystallization and preliminary X-ray crystallographic analysis of recombinant transaldolase B from *Escherichia coli*

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Abstract

Recombinant transaldolase from *Escherichia coli*, an enzyme of the pentose phosphate pathway has been crystallized by the vapor-diffusion method using polyethylene glycol 6000 as precipitant. The crystals are orthorhombic, space group $P2_12_12_1$ with cell dimensions $a = 68.9$, $b = 91.3$ and $c = 130.5$ Å, and diffract to 2 Å resolution on a conventional X-ray source. The asymmetric unit very likely contains two subunits, corresponding to a packing density of 2.9 Å^3 Da^{-1}.

1. Introduction

The pentose phosphate pathway provides D-ribose-5-phosphate for the synthesis of nucleic acids and histidine, D-erythrose-4-phosphate for the synthesis of aromatic amino acids and reducing power in the form of NADPH. In the non-oxidative branch of this pathway, the enzymes transketolase and transaldolase are part of a sugar phosphate rearrangement system and mediate the interconversion of these sugar phosphates through transfer of C2 and C3 ketol fragments, respectively, from ketol donors to appropriate aldose acceptors.

Transaldolase (D-sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate dihydroxyacetonetransferase, E.C. 2.2.1.2) is a ubiquitous enzyme which catalyzes the reversible transfer of a dihydroxyacetone moiety, derived from fructose-6-phosphate to erythrose-4-phosphate yielding sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate (for a review see Tsolas & Horecker, 1972). The reaction proceeds through a covalent intermediate, the transaldolase-dihydroxyacetone complex (Venkatamaran & Racker, 1961). Most likely, this intermediate is a Schiff base between a lysine residue and the dihydroxyacetone moiety (Tsolas & Horecker, 1972; Miosga, Schaaff-Gerstenschläger, Franken & Zimmermann, 1993). This mechanism identifies transaldolase as a member of the class I aldolase family with fructose bisphosphate aldolase as a prominent representative (Littlechild & Watson, 1993). Transaldolase is a rather conserved polypeptide with overall sequence identities of more than 50% between prokaryotic and eukaryotic species (Schaaff, Hohmann & Zimmermann, 1990, Yura *et al.*, 1992, Jacoby, Hollenberg & Heinisch 1993; Banki, Halladay & Perl, 1994). The enzyme from *E. coli* is a homodimer consisting of 316 amino acids per subunit with a calculated M_r of 35 092 (Yura *et al.*, 1992, Sprenger, Schröken, Sprenger & Sahm, unpublished results).

Aldolases are of interest as potential catalysts for stereospecific enzymatic synthesis of carbohydrates and a variety of aldolases have already been used for this purpose (Toone, Simon, Bednarski & Whitesides, 1989). We are interested in exploring the usefulness of transaldolase as catalyst for chemical syntheses and have initiated a three-dimensional structure determination to reveal the molecular architecture of the enzyme and, in particular, its active site. Here, we describe the crystallization of recombinant transaldolase from *E. coli* and report on the crystallographic analysis of these crystals.

2. Materials and methods

2.1. *Enzyme purification*

Transaldolase from recombinant *E. coli* K-12 cells (strain DH5/pGSJ 451) was purified to electrophoretic homogeneity by successive ammonium sulfate precipitation and two anion-exchange chromatography steps. Details of gene cloning and protein purification will be described elsewhere Sprenger, Schröken, Sprenger & Sahm, unpublished results). Enzyme samples (5 mg ml^{-1}) were frozen and stored at 193 K until further use.

Crystallization

Enzyme samples were concentrated to about 20 mg ml^{-1}. Initial screening for crystallization was performed by a simplified version (Jancarik & Kim, 1991) of incomplete factorial experiments (Carter & Carter, 1979) at 277 and 293 K. Crystallization was achieved at 293 K by the hanging-drop vapor-diffusion technique (McPherson, 1976). In the final conditions for crystallization, the reservoir solution contained 0.1 *M* sodium citrate, pH = 4.0, and 15% polyethylene glycol 6000. Protein samples (6 µl) were mixed with equal amounts of reservoir solution and allowed to equilibrate. Crystals grew within 5–7 d, but were usually twinned. To obtain single crystals we employed a combination of micro- and macroseeding. The transaldolase crystals were first crushed into pieces with a glass capillary, and then transferred to another drop set up as above, but where no crystals had yet

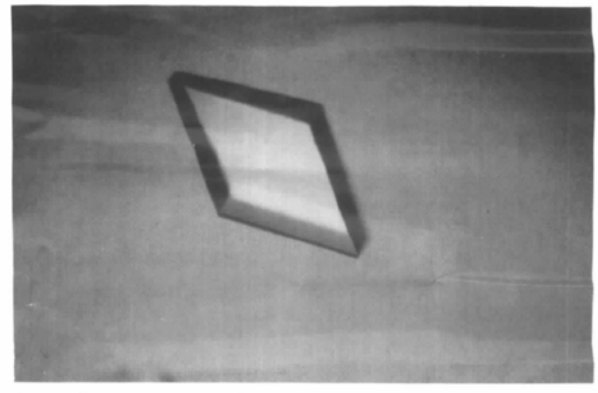

Fig. 1. Crystal of recombinant transaldolase from *Escherichia coli*. The longest dimension is approximately 0.6 mm.

formed. Within a few days, crystals appeared. These crystals were then washed in reservoir solution and again transferred to a new drop of a mixture of protein and reservoir solution. After a few weeks, single crystals grew to about 0.4 × 0.5 × 0.7 mm in size (Fig. 1).

2.2. *X-ray analysis*

Crystals were mounted in thin-walled glass capillaries. A native data set to 2 Å resolution was collected as 1° oscillation frames on an R-AXIS II image-plate detector, mounted on a Rigaku rotating anode, operating at 50 kV and 180 mA and equipped with a graphite monochromator. Data frames were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1993). A partial data set has also been collected at beamline X12-C at the National Synchrotron Light Source, Brookhaven National Laboratory using an MAR image-plate system.

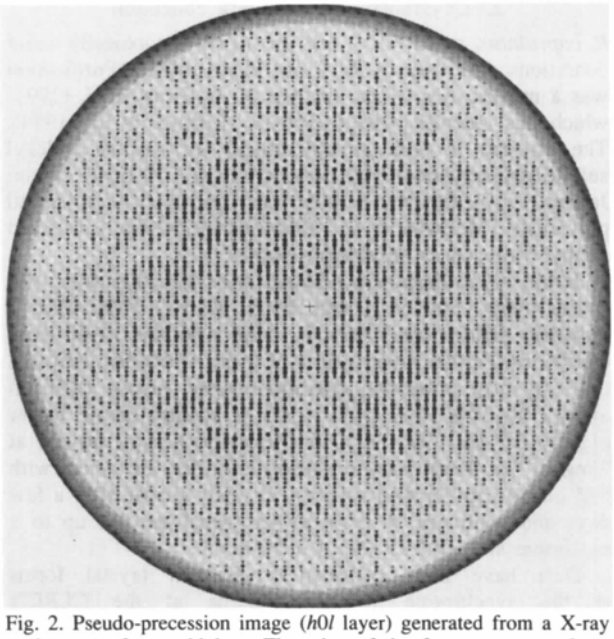

Fig. 2. Pseudo-precession image (*h*0*l* layer) generated from a X-ray data set of transaldolase. The edge of the frame corresponds to 2.0 Å resolution.

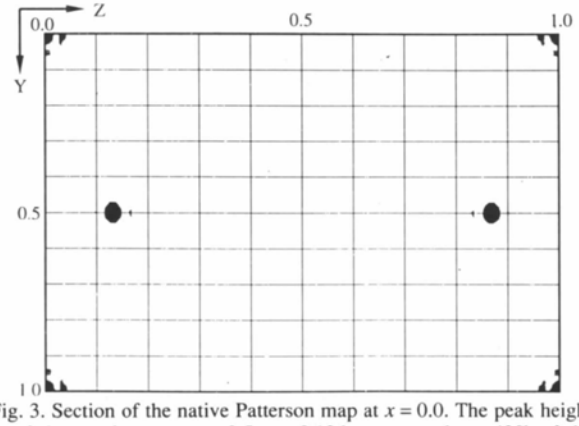

Fig. 3. Section of the native Patterson map at $x = 0.0$. The peak height of the maximum at $y = 0.5$, $z = 0.136$ corresponds to 42% of the origin peak.

3. Results and discussion

Single crystals of transaldolase were obtained by repeated seeding. These crystals are stable in the X-ray beam and diffract to 2.0 Å resolution on a conventional X-ray source. Preliminary experiments at beamline X12-C at NSLS, Brookhaven, show that the diffraction pattern extends to at least 1.8 Å resolution. A native data set to 2 Å resolution was collected on a conventional X-ray source. Indexing by the autoindexing routine in *DENZO* and analysis of the diffraction pattern by pseudo-precession images (Fig. 2) using the program *PATTERN* (Guoguang Lu, unpublished work) are consistent with an orthorhombic space group, $P2_12_12_1$ and cell dimensions $a = 68.9$, $b = 91.3$ and $c = 130.5$ Å. Data processing gave an R_{merge} of 6.4% (based on intensities) and this data set is 84% complete to 2.0 Å resolution (51% completeness in the resolution interval 2.1–2.0 Å).

Given a molecular weight of 35 000, packing densities which would fall in the range usually encountered in proteins (Matthews, 1968) are only obtained for $n = 2$ (2.9 Å^3 Da^{-1}) and $n = 3$ (1.9 Å^3 Da^{-1}), respectively. The native Patterson map, calculated in the resolution range 50–3.0 Å contains a strong maximum (42% of the origin peak) at $x = 0.0$, $y = 0.5$, and $z = 0.136$ (Fig. 3). This indicates that the asymmetric unit of the transaldolase crystals contains two subunits, related by a translational symmetry along y and z.

In conclusion, crystals of transaldolase suitable for a high-resolution structure analysis have been obtained and screening for heavy-atom derivatives is well underway.

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