Acta Cryst. (1998). D**54**, 467–469

Polymorphous crystallization and diffraction of threonine deaminase from *Escherichia coli*

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(*Received 3 June 1997; accepted 21 August 1997*)

Abstract

The biosynthetic threonine deaminase from *Escherichia coli*, an allosteric tetramer with key regulatory functions, has been crystallized in several crystal forms. Two distinct forms, both belonging to either space group $P3_121$ or $P3_221$, with different sized asymmetric units that both contain a tetramer, grow under identical conditions. Diffraction data sets to 2.8 Å resolution (native) and 2.9 Å resolution (isomorphous uranyl derivative) have been collected from a third crystal form in space group $I222$.

1. Introduction

Regulation of the synthesis of the branched-chain amino acids valine, leucine and isoleucine in *Eschericia coli* is accomplished in part by threonine deaminase (TD) (E.C. 4.2.1.16). TD, one of the first allosteric enzymes (Umbarger, 1956, 1992) to be discovered, is composed of four identical polypeptide chains to give a tetrameric quaternary structure of M_r = 220 000. The enzyme catalyzes the pyridoxal phosphate (PLP) dependent α,β-dehydration/elimination of threonine to produce α-keto butyrate, the first step toward isoleucine biosynthesis, in a controlled manner. Enzyme activity is increased by valine and threonine (the former, along with leucine, is produced by a parallel biosynthetic pathway), and is inhibited by isoleucine (Eisenstein, 1991). Biosynthetic TD is distinct from, yet weakly homologous to, a biodegradative threonine dehydratase also present in *E. coli*. Additionally, TD is homologous in part to the PLP-dependent β subunit of tryptophan synthase, whose structure has been determined (Hyde *et al.*, 1988). Previous studies on TD have focused on cooperative ligand binding and feedback regulation (Umbarger, 1992; Eisenstein, 1991; Eisenstein *et al.*, 1994), with an interest in identifying potential applications in the synthesis of plastics precursors (Poirier *et al.*, 1995; Eschenlauer *et al.*, 1996) and in the possibility of inhibiting the enzyme to control microorganisms or weeds, since it belongs to a biosynthetic pathway completely absent in animals.

Several allosteric enzymes have been analyzed by X-ray crystallography, including hemoglobin (Perutz, 1989), aspartate transcarbamylase from *E. coli* (Gouaux *et al.*, 1990), glycogen phosphorylase from rabbit muscle (Johnson & Barford, 1990), phosphofructokinase from *B. stearothermophilus* and *E. coli* (Schirmer & Evans, 1990), and 1,6-bisphosphatase from pig kidney (Ke *et al.*, 1990). Analysis of these proteins provides some insight into general principles governing allosteric control (Perutz, 1989). These proteins function as symmetric oligomers, whose allosteric transitions involve a quaternary rearrangement of subunits. In addition to control by feedback inhibition, these oligomers also demonstrate a sigmoidal increase in activity with increasing substrate concentrations. The sigmoidal kinetics of biosynthetic TD are shifted in the presence of the modifiers isoleucine and valine, which respectively increase and decrease the apparent cooperativity of the enzyme.

Protein structure studies in multiple crystal polymorphs can enhance understanding of the crystal-growth process by comparison of the alternative contact arrangements. Such studies also provide information on protein plasticity, which in the present case may bear on the allosteric transition. In addition, having several crystal forms increases the chance that the allosteric states and ligand complexes of interest can be observed by diffraction. We report three diffraction-quality crystal forms of this enzyme and an isomorphous anomalous-scattering derivative in one of the forms.

2. Materials and methods

Enzyme was purified according to Eisenstein (1991) and concentrated to 30 mg ml^{-1} in 50 m*M* potassium phosphate, pH 7.5, 0.1 m*M* dithiothreitol, 0.1 m*M* ethylene diamine tetraacetic acid. After initial crystals of inadequate size and quality were obtained from ammonium sulfate as precipitant, a sparse-matrix-search method was applied (Jankarik & Kim, 1991), using hanging drops (McPherson, 1995). Use of the Crystal Screen 1, obtained from Hampton Research,† led to discovery of all the crystal forms described below. Trials that produced heavy precipitate were repeated using a dilution of the crystallization reagent. For trials that produced no crystals or precipitate, 5–10 µl of saturated ammonium sulfate was added to the well, to further dehydrate the drop. Diffraction-quality crystals were obtained from two different conditions, both at room temperature. Condition 1 (derived from kit reagent 33) is 4.1 *M* Na formate, pH 7–8, and condition 2 (derived from kit reagent 6) is 12% polyethylene glycol (PEG) 4000, 40 m*M* Tris 8.5, 70 m*M* $MgCl_2$, 0.2 *M* KCl. Crystals were propagated by microseeding in condition 2 to 0.5 mm dimensions with growth times of 5–10 d. Condition 2 crystals were gradually brought to pH 7.2 for 5 d soaking in 0.1 m*M* UO_2SO_4, resulting in an isomorphous derivative for structure determination.

Diffraction data were collected at room temperature by a Siemens electronic area detector mounted on a Supper oscillation camera. X-rays were generated by a Rigaku rotating anode operated at 40 kV and 80 mA. The determi-

† Identification of specific instruments and products in this paper is to document the experimental procedure, and does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

nation of crystal orientation and the integration of reflection intensities were performed with the *XENGEN* program system (Howard *et al.*, 1987). Patterson analysis and phase calculations utilized the *PHASES* program suite (Furey & Swaminathan, 1998). Faces of the orthorhombic crystal form were indexed by analysis of diffraction patterns of oriented crystals coupled with photographic measurement of interfacial angles.

3. Results

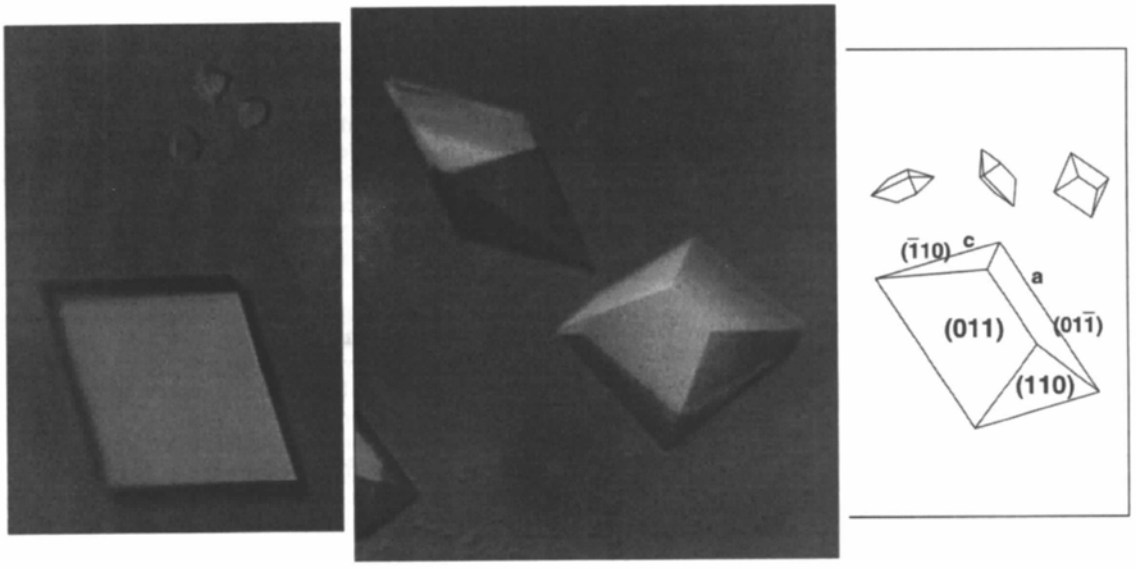
Crystals grown in the formate conditions are shown in Fig. 1(*a*). The two crystal forms differ greatly in size and rate of growth; the larger 'blocks' grow to millimetre dimensions overnight while the small bipyramids take weeks to grow to 0.2 mm. Both crystal forms display threefold symmetry. The 'blocks' are shaped like a rhombohedral unit cell, showing six identical rhombic faces. The bipyramids appear to be hexagonal but on close examination reveal the lower trigonal symmetry. Although the two forms usually grow simultaneously in the same drop, lower pH seems to favor the second bipyramidal form. Both crystal forms belong to space group $P3_121$ or its enantiomorph. The lattice parameters for the 'blocks' are a = 162.5, c = 128.7 Å, while those for the bipyramids are a = 169, c = 183 Å. Matthews parameters (Matthews, 1968) are V_m = 2.2 and 3.4 Å^3 Da^{-1} respectively, assuming a tetramer per asymmetric unit in each form. The room-temperature diffraction limit for these crystal forms appears to be about 2.8 Å.

Crystals obtained using the PEG conditions are shown in Fig. 1(*b*) and drawn in Fig. 1(*c*). These crystals belong to space group *I*222 with one polypeptide per asymmetric unit. The unit-cell dimensions are a = 85.1, b = 90.8, c = 162.5 Å, giving V_m = 2.8 Å^3 Da^{-1}. These crystals diffract to at least 2.3 Å resolution.The simpler packing of these crystals, with the molecular symmetry coinciding with the crystallographic, and the superior diffraction, made this form our first choice for structure determination.

The *I*222 crystals were found to be extremely sensitive to most heavy-metal compounds tested for isomorphous derivatization. Virtually all mercury compounds and many others, at millimolar concentrations, either caused cracking or simply destroyed the birefringence (and diffraction) of the crystals within a few days. Other compounds produced large changes in the unit-cell dimensions and, in a few cases, altered the space group from *I*222 to *P*222. The protein's allosteric mechanism is expected to involve some gross structural rearrangement, which may correlate with these observed crystal instabilities.

Cryodiffraction was attempted with all crystal forms. The sodium-formate-grown crystals of both forms can be flash-frozen without cryoprotectant and diffract about as well as at room temperature. The PEG-4000-grown crystals, however, gradually and reversibly lose diffraction as their temperature is reduced from 297 to 283 K, and lost all diffraction when flash frozen. Cryoprotectants (ethylene glycol, glycerol) enable the crystals to diffract when flash frozen, but with high mosaicity and poor diffraction quality. As a result, all native and derivative data for the structure determination (orthorhombic form) has been obtained at room temperature.

Native data 86% complete to 2.8 Å resolution with R_{sym} = 0.07 were combined with uranyl-derivative anomalous data 91% complete to 2.9 Å with R_{sym} = 0.08. The derivative lattice constants were all within 1% of the native values. The data sets scaled together with R = 14% in the 10–4 Å shell. The anomalous-difference Patterson map (Fig. 2) shows that the uranyl cation binds to the protein at a location with fractional (x,y,z) coordinates (0.114, 0.105, 0.092). Phase calculations utilizing the *PHASES* package (Furey & Swaminathan, 1998)



(*a*) (*b*) (*c*)

Fig. 1. Crystal forms of threonine deaminase: (*a*) two sibling polymorphs growing in a sodium formate solution: three small bipyramids at top, and one large 'block' below; (*b*) crystals of the orthorhombic form, space group *I*222; (*c*) diagram indexing the faces of the orthorhombic form.

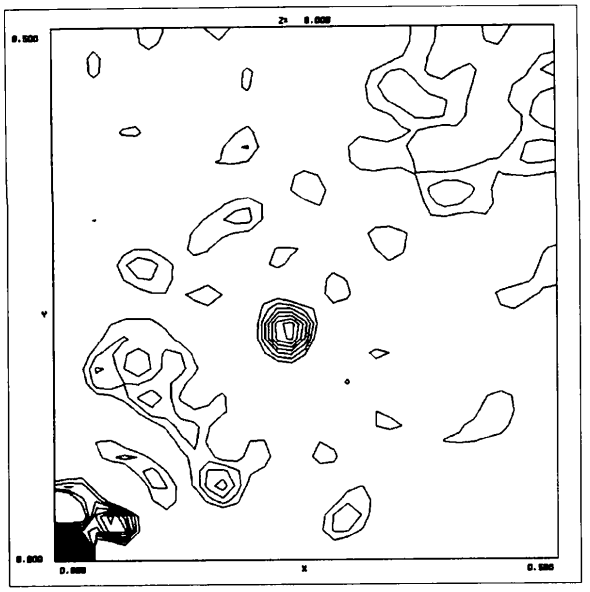


Fig. 2. Anomalous Patterson map (4.5 Å resolution) showing 7σ peak at uranyl site. The z = 0 Harker section is contoured at integer multiples of σ. Maps were calculated using programs from the *PHASES* package (Furey & Swaminathan, 1998).

in the 10.0–3.0 Å shell gave phasing powers of 1.8 and 2.9 for the isomorphous and anomalous contributions, respectively. A single isomorphous replacement plus anomalous scattering (SIRAS) phase set with figure-of-merit 0.70 has been calculated in this resolution shell. Solvent flattening (Wang, 1985) with these phases has produced a map with interpretable protein structure, and model building is in progress.

4. Discussion

Threonine deaminase was one of the first reported allosteric enzymes and it has an extensive background of genetic and biochemical studies. It will therefore be of interest to have a refined crystal structure. Crystal studies to date suggest that the structure is somewhat plastic. This may have some basis in its biological function, which calls for conformational transitions between allosteric states. In particular, the possibility that the two trigonal polymorphs correspond to two distinct protein conformations bears investigation. To account for the enzyme's allosteric transition in terms of identified regulatory binding sites and structural communication is a long-term goal of this work.

This work was supported by grant 962-23-08-37 from NASA/Marshall Space Flight Center.

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