Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of tryptophan synthase α-subunits from *Escherichia coli*

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Received 19 September 2003
Accepted 13 October 2003

Tryptophan synthase α-subunit (αTS) catalyzes the cleavage of indole-3-glycerolphosphate to glyceraldehyde-3-phosphate and indole, which is channelled to the active site of the associated β-subunit (βTS), where it reacts with serine to yield the amino acid tryptophan in tryptophan biosynthesis. The αTS from *Escherichia coli* is a 268 amino-acid protein with no disulfide bonds or prosthetic groups. Although the crystallization of the subunits from *E. coli* has been attempted over many years, there have been no reports of an X-ray structure. To explore the molecular origin of the conformational stabilization mechanism of αTS, the α-subunit protein was overexpressed in *E. coli* and crystallized using the hanging-drop vapour-diffusion method at 298 K. A native data set to 2.8 Å resolution was obtained from a flash-cooled crystal upon exposure to Cu $K\alpha$ X-rays. The crystal belongs to the monoclinic space group $C2$, with unit-cell parameters $a = 162.27$, $b = 44.48$, $c = 71.52$ Å, $\beta = 106.56^\circ$. The asymmetric unit contains two molecules of αTS, giving a crystal volume per protein mass (V_M) of 2.16 Å^3 Da^{-1} and a solvent content of 43.18%.

1. Introduction

Tryptophan synthase has been the subject of many important genetic and biochemical studies; it catalyzes the last reaction in the biosynthesis of L-tryptophan in bacteria, fungi and plants. The enzyme is unique to these organisms and could therefore be a molecular target for the development of novel and selective antibacterial, antifungal or herbicidal agents with limited mammalian toxicity.

The crystal structure of the tryptophan synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* has been determined by X-ray analysis (Hyde *et al.*, 1988). The active sites of neighbouring α- and β-subunits are separated by a distance of 25 Å (Miles, 1995).

The α-subunit alone catalyzes the cleavage of indole-3-glycerolphosphate and the β-subsynthesis of L-tryptophan from indole and L-serine (Creighton, 1970). The overall polypeptide fold of the smaller α-subunit is that of an eightfold α/β-barrel and the larger pyridoxal phosphate-dependent β-subunit contains two domains of nearly equal size folded into similar helix/sheet/helix structures in the $\alpha_2\beta_2$ complex (Hyde *et al.*, 1988).

The $\alpha_2\beta_2$ complex is in an equilibrium between the low-activity 'open' and the high-activity 'closed' state which is shifted by allosteric ligands and monovalent cations (Fan *et al.*, 2000). It is known that ligand-induced intersubunit signals keep the catalytic activities of the α and β active sites in phase (Lane & Kirschner, 1983; Dunn *et al.*, 1990; Anderson *et al.*, 1991; Kirschner *et al.*, 1991). The basis of the corresponding conformational transitions has been characterized by X-ray structure analysis of a number of enzyme–ligand complexes (Rhee *et al.*, 1997; Schneider *et al.*, 1998; Weyand & Schlichting, 1999). In the crystal structures of tryptophan synthase complexed with indole-3-acetylglycine and indole-3-acetyl-L-aspartic acid ligands, ligand binding leads to closure of loop αL6 of the α-subunit and this is in keeping with the allosteric role (Weyand *et al.*, 2002).

Spectral techniques have been used widely as a sensitive means of determining the stability of a protein and to monitor structural transitions such as unfolding and refolding under a variety of conditions. The folding process of αTS from *E. coli* has been studied by various structure-monitoring techniques including fluorescence spectroscopy, UV absorbance, CD and NMR (Bilsel *et al.*, 1999; Lim *et al.*, 1991; Beasty *et al.*, 1986; Ogasahara & Yutani, 1997; Milton *et al.*, 1986).

Although the crystallization of the subunits of tryptophan synthase from *S. typhimurium* and *Escherichia coli* has been attempted over many years (Schultz & Creighton, 1969), there have been no reports of an X-ray structure. Recently, the structure of the αTS from the hyperthermophile *Pyrococcus furiosus* has been studied (Yamagata *et al.*, 2001). The α-subunits from *P. furiosus* and *S. typhimurium* share 31.5% sequence identity. The

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αTS from *P. furiosus* lacked 12 and six residues at the N- and C-termini, respectively, and one residue in each of two loop regions compared with that from *S. typhimurium*. Entropic effects arising from the shortening of the polypeptide chain play important roles in the high stability in *P. furiosus*. It follows that the proteins from hyperthermophiles are unusually stable and form better crystals more easily.

The α-subunits from the mesophiles *S. typhimurium* and *E. coli* are 85% identical in sequence and both α-subunits are stable and stimulate the activity of both β-subunits (Nichols & Yanofsky, 1979). The αTS from *E. coli* consists of a single polypeptide chain of 268 residues (M_r = 28 600). The residues of the α-subunit are disordered when it is bound to the β-subunit to compose mature tryptophan synthase or when the structure is regulated allosterically by ligand binding (Weyand *et al.*, 2002; Wu & Matthews, 2003). We need to study the structure of the α-subunit alone in order to find the conformational changes between αTS and the complex structure in mesophiles. This structural information will suggest the stabilization mechanism and the residues that are involved in the intersubunit communication. The purified α-subunit from *E. coli* produced a suitable crystal for X-ray crystallographic analysis and the crystal structure could be determined to a resolution of 2.8 Å. We report here its overexpression, crystallization and preliminary X-ray crystallographic data.

2. Cloning, overexpression and purification

Plasmid *ptactrpAMK-M13* containing the *trpA* gene was used as an overexpression vector (Sarker & Hardman, 1995) and *E. coli* RB797 (*F′ lacI^q proL8/arg Nal^r Fif^r recA sup lac proXIII*) was used as the host strain for αTS expression vectors (Lim *et al.*, 1991). The *trpA* gene is under control of the *tac* promoter and is inducible by D-lactose (Lim *et al.*, 1991). The cells were grown to an OD_{600} of 0.6 at 310 K and the expression of αTS protein was induced with 1% lactose. The cultured cells were harvested after 24 h induction. Purification of αTS was performed as described elsewhere (Sarker & Hardman, 1995; Kim *et al.*, 2001). The cells were resuspended in SH buffer [10 m*M* potassium phosphate buffer pH 7.8 containing 5 m*M* EDTA, 0.2 m*M* phenylmethylsulfonylfluoride (PMSF) and 10 m*M* β-mercaptoethanol (ME)] and homogenized with an ultrasonic processor (Branson Sonifier model 250). The solution was centrifuged at 15 000 rev min^{-1} for 30 min and the cell debris was discarded. The crude extracts (15 g ml^{-1}) were precipitated by addition of 35–50% saturated ammonium sulfate (100% saturation; 70 g per 100 ml).

Further purification was performed by HPLC (Waters LC Module 1 Plus) using a DEAE (Protein-Pak DEAE 5PW, 21.5 × 150 mm, Waters) column by linear-gradient elution from 10 to 500 m*M* SH buffer. Protein fractions above 95% purity were combined and concentrated by the addition of 85% saturated ammonium sulfate. The pellet was dissolved in buffer *F* (10 m*M* potassium phosphate buffer pH 7.8, 0.2 m*M* EDTA and 1 m*M* β-ME) and was dialyzed against this buffer. Each purified protein appeared as a single band on SDS–PAGE (Laemmli, 1970). The protein concentration of the crude extracts was estimated by the microbiuret assay (Itzhaki & Gill, 1964). The concentration of purified α-subunits was measured by extinction values using $E^{1\%}_{278\text{nm}}$ = 4.4 for the α-subunit (Adachi *et al.*, 1974). The protein solution was concentrated using a Vivaspin 20 Polyethersulfone membrane (10 000 MWCO) to about 10 mg ml^{-1}. The purified αTS was at least 95% pure as judged on a polyacrylamide gel.

3. Crystallization of αTS

Crystals of αTS were obtained by the hanging-drop vapour-diffusion method at 298 K using 24-well Linbro plates (Hampton Research). A hanging drop was prepared by mixing equal volumes (1.0 µl each) of protein solution and reservoir solution. Each hanging drop was placed over 0.5 ml reservoir solution. Initial crystallization conditions were established by sparse-matrix sampling (Jancarik & Kim, 1991). The αTS crystallized as clusters of plate-shaped crystals using a precipitant containing 0.5 *M* ammonium sulfate, 0.1 *M* trisodium citrate dihydrate and 1.0 *M* lithium sulfate monohydrate pH 5.6. Under these conditions, crystals appeared after about 7–10 d and grew to maximum size within two weeks. The crystals grow as extended plates or lathes as either a small single crystal or clusters. The thin dimension of the crystals results in their being quite fragile and difficult to handle. Truncated crystals were transferred to the same buffer in order to pick up a single crystal using a mounted cryoloop for data collection. The crystals obtained were thin plates with typical dimensions of 0.05–1 × 0.2–0.4 × 0.3–0.5 mm (Fig. 1)

Figure 1
Truncated plate crystal forms of tryptophan synthase α-subunits from *E. coli*.

Table 1
Crystal information and data-collection statistics.

Values in parentheses are for the highest resolution bin.

Space group	*C*2
Unit-cell parameters (Å, °)	*a* = 162.27, *b* = 44.48, *c* = 71.52, β = 106.56
Resolution (Å)	30.0–2.8 (2.90–2.80)
Completeness (%)	90.8 (90.8)
Observed reflections	123308
Unique reflections	12425
$I/\sigma(I)$	15.1 (4.9)
R_{merge}† (%)	13.9 (32.1)

† $R_{\text{merge}} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h, i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h, i)$ for all i measurements.

4. Data collection and analysis

Data were collected from a flash-cooled crystal at 100 K using Cu *Kα* X-rays on a Rigaku R-AXIS IV image-plate detector at Pohang University of Science and Technology, South Korea. Prior to data collection, the crystal was soaked briefly in a cryoprotectant solution consisting of precipitant solution containing 20% glycerol. Diffraction data (Table 1) were obtained and processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Native diffraction data were collected to 2.8 Å resolution at 100 K. A total of 123 308 measured reflections were merged into 12 425 unique reflections with an R_{merge} (on intensity) of 13.9%. The merged data set is 90.8% complete to 2.8 Å resolution. The crystal belongs to the monoclinic space group *C*2; unit-cell parameters are given in Table 1. The asymmetric unit contains two molecules of αTS, giving a crystal volume per protein mass (V_M) of 2.16 Å^3 Da^{-1} and a solvent content of 43.18%. Understanding the molecular origin of the conformational stabilization mechanism of αTS from *E. coli* will provide valuable insights into protein stability, folding properties and protein engineering. Table 1 summarizes the statistics of the data collection. We are planning

to use molecular replacement or the multiple isomorphous replacement method to solve the structure.

We thank Professor Byung-Ha Oh, Pohang University of Science and Technology for providing the X-ray equipment. This work was supported by Korea Research Foundation Grant (KRF-2002-041-C00244) and by Pusan National University Research Grant. MSJ was supported by grant No. R03-2002-000-00007-0 from the Basic Research Program of the Korea Science and Engineering Foundation.

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