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Purification and preliminary X-ray crystallographic studies of recombinant 7,8-diaminopelargonic acid synthase from *Escherichia coli*

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Abstract

Recombinant 7,8-diaminopelargonic acid synthase from *Escherichia coli*, a pyridoxal-phosphate-dependent aminotransferase, has been crystallized in space groups $P2_1$ and $C2$. Both crystal forms were obtained at pH 7.3 with 21% polyethylene glycol and 10% 2-propanol as precipitants. The cell dimensions were a = 130, b = 57.5, c = 117 Å, β = 110° for the $C2$ crystals, and a = 58.4, b = 55.6, c = 121 Å, β = 96.9° for the $P2_1$ crystals, which diffract to at least 2.6 and 2.0 Å resolution, respectively.

1. Introduction

Biotin is the cofactor in many biological carboxylation reactions. It is synthesized from pimeloyl coenzyme A in microorganisms and plants. The genes involved in biotin synthesis as well as the corresponding proteins have been characterized in different microorganisms and in higher plants (Baldet *et al.*, 1993; Gloeckler *et al.*, 1990). In *E. coli*, most of the genes encoding the enzymes involved in biotin biosynthesis are collected in a gene cluster, the *bio* operon (Eisenberg, 1985). This operon, which contains five genes *bio A*, *B*, *F*, *C* and *D*, has been sequenced and the amino-acid composition of the encoded enzymes predicted. The five proteins have been characterized to various extents (Otsaku *et al.*, 1988; Eisenberg, 1985).

The *bioA* gene product, 7,8-diaminopelargonic acid synthase (DAPA synthase), catalyses the antepenultimate step in the biotin biosynthesis, the conversion of 7-keto-8-aminopelargonic acid (KAPA) to 7,8-diaminopelargonic acid (DAPA). The enzyme is a pyridoxal-phosphate-dependent aminotransferase with the rare feature that it uses *S*-adenosyl methionine as amino-group donor. However, kinetic studies indicate that it uses a bi–bi ping-pong mechanism, as is typical for aminotransferases. DAPA synthase is a homodimer with a molecular mass of 94 kDa (Stoner & Eisenberg, 1975*b*). The primary structure predicted from the gene sequence consists of 430 residues per monomer (Otsaku *et al.*, 1988).

We are focusing on structural characterization of enzymes in the biotin synthesis pathway and have earlier reported the structures of the penultimate enzyme, dethiobiotin synthetase, with several complexes and their mechanistic implications (Huang *et al.*, 1994, 1995; Gibson *et al.*, 1995). Here we report overexpression, purification and initial crystallographic studies of DAPA synthase.

2. Cloning and expression of *bioA* in *E. coli*

A DNA fragment encoding DAPA synthase was placed under transcriptional control of the bacteriophage T7 promoter. Plasmid pXL1606 had been constructed to drive expression from the *trp* promoter, with translation ensured by the fusion of the *bioA* initiator methionine to the ribosome binding site from gene 10 of bacteriophage T7 (Lévy-Schil *et al.*, 1993). Plasmid pXL1606 was digested with restriction enzymes *Eco*RI and *Hin*dIII and a 2.5 kb fragment containing *Ptrp*, gene 10 rbs and *bioA* isolated from low-melting-point agarose gel. The purified fragment was ligated into the *Eco*RI and *Hin*dIII sites of the Novagen T7 expression plasmid pET24(+). The T7 RNA polymerase producing strain BL21(DE3) was transformed with the resulting construct (Studier & Moffat, 1986). Transformants were selected on LB plates containing 50 mg l^{-1} kanamycin, screened by digesting miniprep DNA with *Eco*RI and *Hin*dIII, and a positive clone purified and designated pT7bioA.

3. Purification of DAPA synthase

DAPA synthase was purified from BL21(DE3)/pT7bioA by modification of the method developed by Stoner & Eisenberg (1975*a*). Fermentations (10 l) were carried out essentially as described for the corresponding dethiobiotin synthase overproducer (Gibson *et al.*, 1995). Cell paste (100 g) was suspended in HEM buffer (50 m*M* HEPES–NaOH, pH 7.5, 0.1 m*M* EDTA and 10 m*M* 2-mercaptoethanol), and passed through a French press at 110 MPa. Debris was removed by centrifugation (12 000*g*, 15 min), and washed with HEM (400 ml). The two supernatants were combined, and the protein concentration was brought to 6 mg ml^{-1} with HEM.

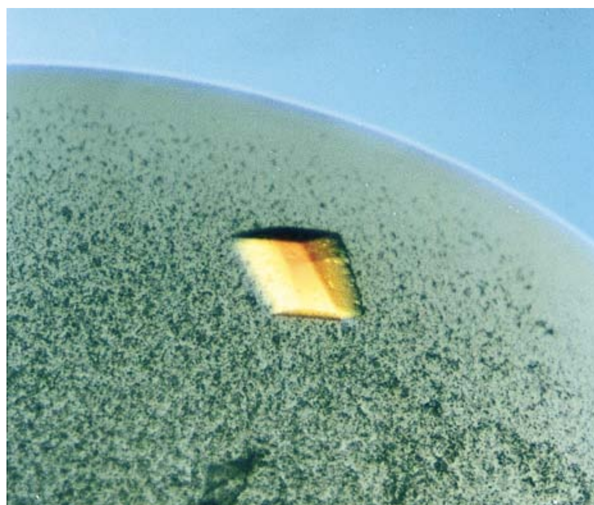

Fig. 1. A crystal of 7,8-diaminopelargonic acid synthase.

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The combined supernatants were supplemented with streptomycin sulfate (5 g, in 50 ml water) and pyridoxal phosphate (final concentration, 0.2 m*M*). The extract was quickly heated to 330 K in a boiling water bath, placed in a 330 K water bath for 15 min, and then chilled. Precipitated protein and nucleic acid were removed by centrifugation, and the supernatant was brought to pH 5 with acetic acid, and allowed to stand on ice for 20 min. Precipitate was removed by centrifugation, and the supernatant was titrated to pH 7.5 with NaOH. The sample was then applied to Q-Sepharose (350 ml; Pharmacia) in TB (50 m*M* Tris–HCl, pH 8, plus 10 m*M* 2-mercaptoethanol), and eluted using a linear gradient (2 l each TB, TB and 1 *M* NaCl). The protein was desalted by dilution in TB and reconcentration over a semipermeable membrane (YM-30, Amicon). The procedure typically gave enzyme that appeared to be 85–90% pure on sodium dodecyl sulfate gels. Attempts to improve purification with additional chromatographic steps (gel filtration, phenyl Sepharose, various dye columns) were unrewarding.

4. Crystallization and crystallographic analysis

Crystallization was carried out at room temperature using the vapour-diffusion technique. 5 µl protein solution (10 mg ml^{-1}) was mixed with an equal volume of reservoir solution. The initial crystallization trials were performed using a commercially available simplified version (Jancarik & Kim, 1991) of incomplete factorial crystallization experiments (Carter & Carter, 1979). Crystals occurred after some weeks under three different sets of conditions, one of which gave crystals that were reproducible. After refinement of these conditions, X-ray grade crystals were obtained at pH 7.3 with a reservoir solution containing 21% PEG 4000 and 12% 2-propanol as precipitants. A typical crystal size is 0.2 × 0.2 × 0.2 mm (Fig. 1). The crystallographic analysis of these crystals revealed that two crystal forms can occur in the same droplet. DAPA synthase crystals were successfully frozen by addition of 15% 2-methyl-2,4-pentanediol (MPD) as cryoprotectant. Use of 20% MPD instead of 2-propanol in the crystallization resulted in crystals which could be mounted and frozen directly from the droplet. In the case of the $P2_1$ crystals, the resolution of the observable diffraction pattern increased by approximately 1 Å upon freezing.

Crystals were mounted on a Rikagu rotating-anode operating at 50 kV, 90 mA. A data set was collected on a MAR image-plate detector to a resolution of 2.6 Å. The data, collected on two crystals, were indexed, processed and scaled using the *DENZO* and *SCALEPACK* software (Otwinowski, 1993). Space-group determination was carried out using the autoindexing routine in *DENZO* (Otwinowski, 1993) and by inspection of the pseudo-precession images generated with the program *Pattern* (Guoguang Lu, unpublished). The crystals belonged to space group $C2$ with cell dimensions a = 130, b = 57.5, c = 117 Å and $\beta = 110°$. The data set was complete to 98.3% (95.6% for the highest resolution shell; 2.69–2.60 Å), had an R_{merge} of 9.4% (36.8%) and an overall multiplicity of 3.6.

A second native data set was collected on a frozen (100 K) crystal at beamline X11, EMBL in Hamburg, on a MAR detector. The data set, processed with *DENZO* and *SCALEPACK* (Otwinowski, 1993), was 98.1% complete to 2.0 Å (97.3% for the 2.07–2.0 Å resolution shell), had an R_{merge} of 6.6% (23%) and an overall redundancy of 2.6. The space-group determination, performed as above, showed that the crystal belonged to space group $P2_1$, with cell dimensions a = 58.4, b = 55.6, c = 121 Å and $\beta = 96.9°$.

Both space groups have almost equally large asymmetric units. Packing involving a dimer in the asymmetric unit would give a packing density of 2.2 $Å^3\,Da^{-1}$. Any other packing yields densities which fall outside the empirical range 1.68–3.53 $Å^3\,Da^{-1}$ found by Matthews (1968). A rotation function, calculated with the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994), showed no significant peak except for the crystallographic twofold axis for data from either space group. Native Patterson maps show no peak for the $C2$ crystal form, while in the $P2_1$ case a peak at position (0.5, 0.5, 0.5) is found. The height of the peak is 6% of the origin peak (8.7σ). Transition from one crystal form to the other, *i.e.* $C2$ to $P2_1$, has been observed experimentally. The change was induced by cooling the crystal from room temperature to 277 K.

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

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