Crystallization and preliminary X-ray crystallographic data of the bifunctional enzyme phosphoribosyl-anthranilate isomerase–indole-3-glycerol-phosphate synthase from *Escherichia coli*

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Received 23 August 1982

Phosphoribosyl-anthranilate isomerase : indole–3-glycerol-phosphate-synthase, a monomeric bifunctional enzyme of M_r = 49 500 from *Escherichia coli*, catalyses 2 sequential reactions in the synthesis of tryptophan from chorismate. Its amino acid sequence, as predicted from the gene sequence, comprises 452 amino acids. Crystals in space group $P4_1$ (or $P4_3$) have been grown from ammonium sulphate, and in $P4_12_12$ (or $P4_32_12$) from polyethyleneglycol, by vapour diffusion and seeding techniques. Three heavy-atom derivatives of the $P4_1$ form with cell dimensions a = 104.7 Å and c = 67.7 Å have been prepared. A structure determination is underway.

Multifunctional enzyme *Phosphoribosyl-anthranilate isomerase* *Indole-3-glycerol-phosphate synthase* *Crystallisation* *Repeated seeding* *X-ray crystallography*

1. INTRODUCTION

The bifunctional enzyme phosphoribosyl-anthranilate isomerase–indole-3-glycerol-phosphate synthase (PRAI–InGPS) of *Escherichia coli* is the second in a series of complex enzymes involved in the biosynthesis of tryptophan from chorismate [1,2]. In *E. coli* and other enteric bacteria [3] it is a monomeric enzyme, a single polypeptide chain catalyzing 2 sequential reactions: the Amadori rearrangement of PRA to CdRP, and the ring closure of CdRP to InGP. In *Bacillus subtilis* and *Pseudomonas putida* the reactions are catalyzed by 2 separate enzymes [4–6] while in *Brevibacterium flavum* they form a multienzyme complex [7]. Such diversity of organization has prompted the suggestion that evolution may have progressed from single function enzymes via multienzyme complexes to multifunctional enzymes [8]. Limited proteolysis studies with *E. coli* PRAI–InGPS have shown that an N-terminal fragment catalyzes the InGPS reaction, while a C-terminal fragment independently catalyzes the PRAI reaction [9]. The enzyme is coded by the *trpC* gene in the tryptophan operon of *E. coli*. This gene has been sequenced [10]. The predicted protein sequence comprises 452 amino acids, corresponding to M_r 49 500.

Here, the preparation of large crystals of PRAI–InGPS via the repeated seeding technique is reported and the preliminary crystallographic data are given for 2 crystal forms of InGPS–PRAI.

Abbreviations: PRA, *N*-(5′-phospho-D-ribosyl)-anthranilate; PRAI, PRA isomerase; InGP, 1-C-(3′-indolyl)-glycerol-3-phosphate; InGPS, InGP synthase (EC 4.1.1.48); CdRP, 1-(2′-carboxyphenylamino)-1-deoxy-ribulose-5-phosphate; PEG, polyethyleneglycol; DTE, dithioerythritol; M_r, relative molecular mass

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Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

2. MATERIALS AND METHODS

The enzyme was purified and microcrystalline material was prepared as in [11].

For crystallization experiments the microcrystalline PRAI–InGPS was dissolved and dialyzed at 4°C against 50 mM potassium phosphate, 5 mM EDTA, 2 mM DTE (pH 6.8). The presence of 25–50 mM phosphate was found to be essential for the production of good crystals. Two different precipitating agents have yielded 2 different crystal forms. Both were initially grown by vapour diffusion at room temperature using the hanging drop method, and later by both hanging and sitting drop vapour diffusion and application of the macro-seeding technique [12].

For purposes of space group determination and heavy atom derivative screening, photographs were taken with a precession camera (Enraf-Nonius, Delft). The radiation source was an Elliott GX6 rotating anode generator (Elliott-Automation Radar Systems, Neutron Div., Borehamwood), which was operated at 40 kV and 60–70 mA, the focal spot on the anode being 0.3 mm × 3 mm.

Precession films were scanned with an optical densitometer (Optronics Scanner P1000, Optronics International Inc., Chelmsford MA) and data reduction was performed on a Digital PDP 11/45 computer.

3. RESULTS AND DISCUSSION

3.1. *Crystallization and crystal data*

Type I crystals were grown from a solution of 5–10 mg enzyme/ml in 0.8 M ammonium sulphate, 50 mM potassium phosphate (pH 7.5), 5 mM EDTA and 1–2 mM DTE by vapour-difusion against 1.6 M ammonium sulphate in the same buffer. The thin long needles (~0.01 mm diam.) obtained are not suitable for X-ray analysis. The needles were therefore cut into 0.2 mm long pieces, washed in 1.3 M ammonium sulphate and reseeded in 60 μl drops containing 4 mg enzyme/ml in the same buffered 0.8 M ammonium sulfate solution. The drops are equilibrated against 1.3 M ammonium sulfate in the same buffer. The reseeding process is repeated every 2–3 days. After a total of ~10–20 seedings, crystals with dimensions

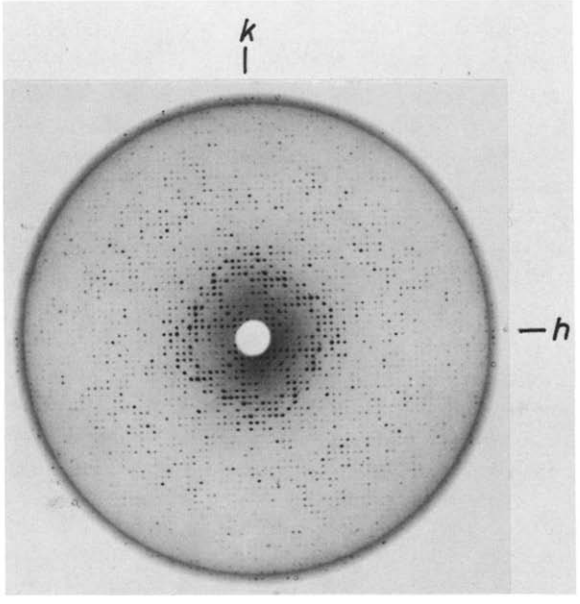

Fig.1a. *hko* projection of the tetragonal crystal having space group $P4_1$ or enantiomorph. The precession photograph (μ = 16°) was exposed for 63 h using graphite monochromated radiation (as described in text) and a crystal-to-film distance of 75 mm.

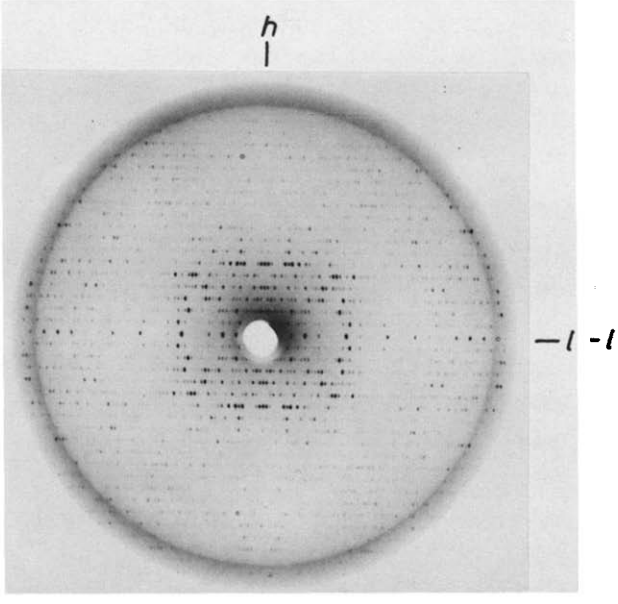

Fig.1b. *hol* projection of the tetragonal crystal having space group $P4_12_12$ or enantiomorph. The precession photograph (μ = 13°) was exposed for 50 h using graphite monochromated radiation and a crystal-to-film distance of 100 mm.

up to 0.5 mm × 0.5 mm × 0.8 mm can be obtained. The crystals belong to space group $P4_1$ or enantiomorph with cell dimensions $a = b = 104.7$ Å and $c = 67.7$ Å. There is 1 molecule of M_r 49 500/asymmetric unit with a solvent content parameter V_M of 3.75 Å^3/dalton. This value falls within the range commonly observed for protein crystals [13]. A precession photograph is shown in fig.1a.

Type II crystals are tetragonal bipyramids. They were grown using the hanging drop method from solutions of 5 mg enzyme/ml, 50 mM potassium phosphate (pH 6.8), 5 mM EDTA, 1–2 mM DTE and 16–18% (w/v) PEG 10 000, equilibrated against 32–36% (w/v) PEG 10 000 in the reservoir. Once or twice reseeding the crystals from this experiment yielded crystals of up to 0.5 mm in the smallest dimension. These crystals belong to space group $P4_12_12$ or enantiomorph with cell dimensions $a = b = 69.7$ Å, $c = 240.7$ Å. The V_m for this crystal form is 2.95 Å^3/dalton. A precession photograph is shown in fig.1b. For both crystal forms diffraction patterns extend to Bragg spacings of at least 2.5 Å on oscillation photographs.

3.2. *Data collection and cooling of crystals*

Both crystal forms of PRAI-InGPS suffer from severe radiation damage, probably due to their large solvent content and loose packing. In the resolution range 2.5–3 Å the crystals diffract very weakly and after 10–15 h exposure in the X-ray beam (graphite monochromated radiation of 40 kV and 40 mA generated by an Elliott GX-6 rotating anode generator) no reflections are observed in this resolution range. Cooling the crystals to 4°C increased their life time in the beam by a factor of 2. An additional marked improvement in the quality of the precession and oscillation photographs was achieved through optimization of the collimation geometry according to [14] combined with an increase of the focal spot size on the GX-6 generator: 3.0 mm × 0.3 mm, 3.0 kW *vs* 2.0 mm × 0.2 mm, 1.6 kW.

The $P4_1$ crystal form is more suitable for a structure determination due to its smaller unit cell dimensions. A 3.4 Å resolution diffractometer data set has been collected at room temperature and we have started to collect a native oscillation data set to 2.5 Å resolution at 4°C.

3.3. *The preparation of heavy atom derivatives*

The preparation of heavy atom derivatives of PRAI-InGPS crystals initially was not very successful. The crystals cracked very easily when soaked in heavy atom solutions and often the crystalline order was completely destroyed. However, we were able to produce good precession photographs of the *nko* zone of derivative crystals with observable intensity changes by using soaking times of only one to a few hours. We checked the derivatives by means of precession photographs of the centric *hko* projection. Useful derivatives were obtained with the compounds $K_2Pt(CN)_4$, $KAu(CN)_2$ and thiomersalyic acid. Difference patterson maps in projection between the 3 derivative- and the native projection data sets were calculated and interpreted. Two sites in the $KAu(CN)_2$, 1 site in the thiomersalyic acid and 2 sites in the $K_2Pt(CN)_4$ derivative were found and refined. Three-dimensional data sets of these derivatives are currently being collected to 3.4 Å at room temperature on a diffractometer and to 3–2.5 Å at 4°C on an oscillation camera.

4. DISCUSSION

PRAI-InGPS is to our knowledge the first multifunctional enzyme studied by X-ray crystallography. The three-dimensional structure of this enzyme will open the possibility to study the mechanism of catalysis of two chemical reactions: the Amadori rearrangement catalysed by the isomerase and the ring-closure reaction catalysed by the synthase. Kinetic studies [15] have shown that the 2 active sites are different and independent. We also know from limited proteolysis studies [9] that the 2 functions are located on different autonomously folding domains and that the N-terminal domain alone is quite unstable. The selection advantage of the fused state of these 2 enzymes could be the stabilization of the N-terminal domain. The basis for this stabilization should become obvious when the three-dimensional structure of this bifunctional enzyme is known.

The bifunctional enzyme is the first candidate of the well-studied pathway of tryptophan biosynthesis to be amenable to crystallographic study, to our knowledge. It is known (see [8]) that in other related organisms the two enzymatic functions occur either in separate proteins, as a multienzyme

complex or as a multifunctional enzyme having in addition a third enzymatic function. Since the DNA sequences of the homologous enzyme PRAI from *Saccharomyces cerevisiae* [16] and the trifunctional enzyme glutamine amidotransferase: InGPS–PRAI from *Neurospora crassa* (M. Schechtman and C. Yanofsky, personal communication) are available and more sequences will presumably be determined in the near future we should be able to fit these amino acid sequences to the spatial structure of the *E. coli* enzyme and to examine which parts of the tertiary structure are most highly conserved. Thus this structure should provide a useful basis for studying the evolution of enzymes participating in the same pathway.

ACKNOWLEDGEMENTS

This work was supported in part by grant 3.415.78 (to Professor J.N. Jansonius) and 3.744.80 (to Professor K. Kirschner) of the Swiss National Science Foundation. We thank Halina Szadkowsky for preparing the enzyme, Mrs Balsiger for typing and Dr M.G. Vincent for reading this manuscript.

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