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Crystallization and preliminary X-ray analysis of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (tyrosine inhibitable) from *Saccharomyces cerevisiae*

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3-Deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (E.C. 4.1.2.15) catalyzes the first step in the biosynthesis of aromatic amino acids: the condensation of phophoenolpyruvate and erythrose 4-phosphate to 3-deoxy-D-*arabino*-heptulosonate-7-phosphate. Diffraction-quality crystals of the tyrosine-inhibitable form of the enzyme from *Saccharomyces cerevisiae* have been obtained by the hanging-drop vapour-diffusion method in the presence of polyethylene glycol. The crystals belong to the triclinic space group $P1$, with unit-cell parameters $a = 81.5$, $b = 94.0$, $c = 104.6$ Å, $\alpha = 65.5$, $\beta = 85.2$, $\gamma = 75.0^\circ$, and can be flash-cooled using glycerol as a cryoprotectant. A data set to 2.3 Å has been collected at 120 K.

1. Introduction

Biosynthesis of the three aromatic amino acids in microorganisms and plants starts with the production of their common precursor chorismic acid as a result of the shikimate pathway. The first reaction of this pathway, the condensation of erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP) to 3-deoxy-D-*arabino*-heptulosonate-7-phosphate, is catalyzed by 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (DAHPS; E.C. 4.1.2.15).

The yeast *Saccharomyces cervisiae* contains two DAHPS isoenzymes whose activity is subject to a twofold regulation. Amino-acid starvation increases enzyme synthesis *via* the activator Gcn4p, which increases transcription of the corresponding genes (Künzler *et al.*, 1992). At the enzyme-activity level, the final products of the pathway, phenylalanine and tyrosine, specifically regulate the isoenzyme acitivities (Paravicini *et al.*, 1989; Schnappauf *et al.*, 1998); the DAHPS isoenzyme encoded by the *ARO3* gene is regulated by phenylalanine and the *ARO4*-encoded isoenzyme is inhibited by tyrosine. A wide variety of differentially controlled isoenzyme species has been observed for DAHP synthases in other organisms (Byng *et al.*, 1982; Byng & Jensen, 1983). Other organisms, including *Escherichia coli* or *Neurospora crassa*, carry a third DAHPS which is inhibited by tryptophan (Hoffmann *et al.*, 1972; Nimmo & Coggins, 1981*a*,*b*; Byng & Jensen, 1983).

Crystallization of the phenylalanine-regulated DAHPS from the prokaryote *E. coli* has been reported recently (Shumilin *et al.*, 1996). Our investigation focuses on the tyrosine-regulated DAHPS from the eukaryote *S. cerevisiae*. The primary sequence deduced from the *ARO4* gene consists of 370 codons, leading to a calculated molecular weight of 39.7 kDa (Künzler *et al.*, 1992). The enzyme requires a divalent metal cofactor for full activity. It is not known which metal is incorporated by *S. cerevisiae in vivo*, but after treatment with the chelating agent EDTA, addition of metal ions such as Co^{2+}, Zn^{2+}, Cu^{2+} and Fe^{2+} restores activity to varying levels (Schnappauf *et al.*, 1998).

Here, we describe a modified purification protocol, crystallization and preliminary X-ray analysis for the tyrosine-inhibitable DAHPS from *S. cerevisiae*.

2. Experimental

2.1. Purification

The tyrosine-inhibitable DAHPS was isolated from *S. cervisiae* strain RH1326 (Mat a, *aro3*-2, *aro4*-1, *leu2*-2, *gcd2*-1) using the multicopy plasmid pME1200 (2 µm, *ARO4*, *LEU2*) and purified as described in Schnappauf *et al.* (1998), with the following modifications. In order to keep the preparation metal-free after removal of the *in vivo* incorporated metal, 0.4 m*M* EDTA was added to all buffers during the chromatography purification steps. At the end of the procedure in Schnappauf *et al.* (1998), an additional anion-exchange chromatography step with 10 m*M* Tris buffer pH 9.0 was applied in order to remove the last traces of contaminant proteins and to shift the system from pH 7.6 to pH 9.0. At a pH value of 9.0, the enzyme is active and the protein solubility is significantly increased (the calculated isoelectric point of DAHPS is 6.5). For a typical preparation, the resulting solution contained the protein at a concentration between 0.3 and 0.5 mg ml^{-1} as determined by the method of Bradford (1976) and was incubated with $ZnCl_2$

at five times the enzyme concentration for 4 h at 277 K. After addition of PEP (to five times the enzyme concentration), the solution was incubated again for 4 h at 277 K. After an additional dialyzation step overnight against 10 m*M* Tris pH 9.0 containing 1 m*M* DTT, the protein solution was concentrated to 18 mg ml^{-1} and stored at 203 K.

2.2. Crystallization

Initial crystallization trials employing the hanging-drop vapour-diffusion method were performed with 41 high-pH solutions from Hampton Crystal Screens I (Jancarik & Kim, 1991) and II (Cudney *et al.*, 1994) at 205 K. Each well of a Sarstedt tissue-culture plate contained 1 ml precipitant buffer and 4 µl of a 1:1 mixture of protein and precipitant buffer hanging on a siliconized cover slip. Within three days, small crystals (~0.05 mm in the longest dimension) appeared in Crystal Screen I condition No. 42 [0.05 *M* KH_2PO_4, 20%(*w*/*v*) PEG 8000] and in Crystal Screen II condition No. 45 [0.01 *M* $NiCl_2$, 0.1 *M* Tris pH 8.5, 20%(*w*/*v*) PEG monomethylether 2000]. Subsequent fine screens, employing identical equilibration conditions, included the use of a wide variety of different molecular-weight PEGs, addition of cations and adjustment of the pH in the range 8.0–9.0 using Tris–HCl buffer.

2.3. Data collection and processing

A rectangular crystal of approximate dimensions 0.50 × 0.20 × 0.10 mm grown from 18% PEG 3000 buffered with 10 m*M* Tris–HCl pH 9.0 was selected and the crystallization drop solution was exchanged for a buffer containing 20% PEG 3000, 10 m*M* Tris–HCl pH 9.0 and 25% glycerol as a cryoprotectant. To reach the final concentration of the cryoprotective agent, the mother liquor of the crystal was exchanged stepwise against solutions containing 5, 10, 15, 20 and 25% glycerol. The crystal was then picked up with a fibre loop (Teng, 1990) and immediately flash-cooled in a stream of gaseous nitrogen at 120 ± 1 K from a home-made cryo-apparatus. A data set was collected on a Siemens HiStar multi-wire proportional counter using graphite-monochromated Cu $K\alpha$ radiation from a Siemens M18X rotating-anode X-ray generator (fine-focus filament, graphite monochromator, 0.5 mm collimator) operated at 50 kV, 90 mA. The data set was obtained using ω and φ scans of 0.25° with 5 min exposure time. To establish the diffraction limit, partially complete data to 2.3 Å were collected using a 30 min exposure time. Reflections were indexed using *FRAMBO* Version 3.003 (Bruker Analytical X-ray Systems) and integrated with *SAINT* Version 5.00 (Bruker Analytical X-ray Systems). A semi-empirical correction for absorption and crystal decay was applied. Symmetry-equivalent reflections intensities were merged using *XPREP* Version 6.00 (Bruker Analytical X-ray Systems).

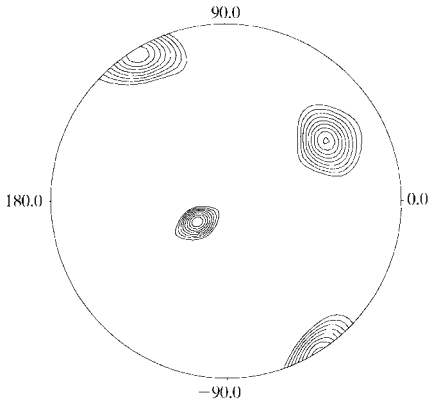

Figure 1
A plot of the self-rotation function generated with *POLARFFN*. Radial coordinate ϖ, angular coordinate φ (anticlockwise). The self-rotation function for reflections between 10.0 and 2.8 Å was evaluated in steps of 2°, with minimum and maximum integration radii of 2.8 and 14.0 Å, respectively. Countouring starts at 55% of the origin peak (corresponding to 2 r.m.s.d.s of the self-rotation function) with intervals of 5%.

2.4. Data analysis

Merged diffraction intensities were converted to structure-factor amplitudes using the program *TRUNCATE* (French & Wilson, 1978; Collaborative Computational Project, Number 4, 1994). The self-rotation function for reflections between 10.0 and 2.8 Å was evaluated in steps of 2°, with minimum and maximum integration radii of 2.8 and 14.0 Å, respectively, using the program *POLARFFN* (Collaborative Computational Project, Number 4, 1994).

3. Results

Crystals of DAHPS have been grown using PEG 2000 monomethylether (Fluka), PEG 3000 (Fluka), PEG 6000 (Hampton Research) and PEG 8000 (Hampton Research) as the precipitant at concentrations between 13 and 18%(*w*/*w*). Crystals grow between pH 8.0 and 9.0, the best results being obtained in 10 m*M* Tris–HCl at pH 9.0. At room temperature, crystals grow to maximum dimensions 0.50 × 0.20 × 0.1 mm within 3 d.

Diffraction data to a resolution of 2.3 Å have been collected from a single crystal of DAHPS at 120 K. From infinity to 2.8 Å, the data are 96.6% complete with an average $I/\sigma(I)$ of 14.4 [90.8% complete and $\langle I/\sigma(I)\rangle$ = 3.9 for the resolution shell 2.90–2.80 Å]. Using prolonged exposure times, diffraction intensities are significant [$\langle I/\sigma(I)\rangle \simeq 2$] to 2.3 Å resolution, but owing to the low crystallographic symmetry of the crystals it was not feasible to collect complete data to this resolution on an in-house source. The crystals belong to the triclinic space group *P*1, with unit-cell parameters a = 81.5, b = 94.0, c = 104.6 Å, α = 65.5, β = 85.2, γ = 75.0°.

The three highest peaks in the self-rotation function are found in the κ = 180° section at (ϖ, φ, κ) = (67.2, 30.4, 180.0°), (157.2, 35.2, 180.0°) and (88.2, 121.1, 180.0°) (Fig. 1). These peaks correspond to three mutually perpendicular (all angles between the direction of maximum self-rotation overlap are 90.0 ± 0.4°) twofold axes, indicating a 222 non-crystallographic symmetry.

Fulfilment of the 222 non-crystallographic symmetry requires the presence of at least four or a multiple of four molecules in the unit cell. The Matthews coefficient V_m (Matthews, 1968) for four molecules of DAHPS in the unit cell is 4.4 Å^3 Da^{-1}. This value corresponds to only 28% of the unit cell being occupied by protein atoms, making it more likely that the unit cell accomodates eight molecules, giving rise to a solvent content of 44%, which corresponds to a V_m of 2.2 Å^3 Da^{-1}.

So far, no structure with a homologous sequence which could be used for molecular replacement has been deposited in the Protein Data Bank. The active-site metal can be readily replaced, and crystallization of DAHPS in complex with different metals is under way. Given the high degree of non-crystallographic symmetry present in the crystals, relatively weak experimental phase information may suffice to solve the structure.

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