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Expression, purification and preliminary crystallographic analysis of phosphoribosyl isomerase (PriA) from *Streptomyces coelicolor*

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The *priA* gene encoding the enzyme phosphoribosyl isomerase from *Streptomyces coelicolor*, a novel bifunctional enzyme involved in both histidine and tryptophan biosynthesis, was heterologously expressed and purified in *Escherichia coli* as an N-terminal His-tag fusion. The purified recombinant enzyme was crystallized using the hanging-drop method in 1.50 *M* ammonium sulfate and 100 m*M* sodium citrate pH 4.8. Crystals were obtained of up to 0.05 × 0.05 × 0.3 mm in size. A full data set to 2 Å resolution was collected at the ESRF beamline ID14-1 and space group $P3_{1,2}21$ was assigned, with unit-cell parameters a = 65.1, c = 104.7 Å.

1. Introduction

Phosphoribosyl isomerase (PriA) is a recently discovered enzyme with a dual function involved in both histidine and tryptophan biosynthesis in the high-(G+C)-content Gram-positive bacteria *Streptomyces coelicolor* and *Mycobacterium tuberculosis* (Barona-Gómez & Hodgson, 2003). The discovery of the dual functionality of PriA arose from the observation that the genomes of *S. coelicolor* and *M. tuberculosis* (Barona-Gómez & Hodgson, 2003), as well as those of several other actinomycetes (Barona-Gómez, 2003), contain no apparent *trpF* gene. It has previously been shown *in vivo* that both HisA and TrpF activity are present in the gene product of *priA* (gene Nos. SCO2050 and Rv1603) using deletion mutants and complementation studies (Barona-Gómez & Hodgson, 2003).

Barona-Gómez & Hodgson (2003) proposed that a broad substrate specificity allows PriA to perform the corresponding Amadori rearrangements of the histidine and tryptophan substrates within these biosynthetic pathways. It appears that these two enzyme functions are more commonly found on independent proteins in most organisms other than actinomycetes (Barona-Gómez & Hodgson, 2003). In histidine biosynthesis, [(5′-phosphoribosyl)-formimino]-5-aminoimidazole-4-carboxamide ribonucleotide isomerase (ProFARI or HisA; EC 5.3.1.16) catalyses the isomerization of *N*′-[(5′-phosphoribosyl)formimino]-5-aminoimidazole-4-carboxamide ribonucleotide (ProFAR) into *N*′-[(5′-phosphoribulosyl)formimino]-5-aminoimidazole-4-carboxamide ribonucleotide (PRFAR), while in tryptophan biosynthesis *N*′-(5′-phosphoribosyl)anthranilate isomerase (PRAI or TrpF; EC 5.3.1.24) catalyses the conversion of *N*′-(5′-phosphoribosyl)anthranilate (PRA) into 1-(2-carboxyphenylamino)-1-deoxyribulose 5-phosphate (CdRP). Although HisA and TrpF are not interchangeable between these biosynthetic pathways, it has been shown that both reactions have similar mechanisms involving general acid–base catalysis and a Schiff-base intermediate (Henn-Sax *et al.*, 2002). The results of Barona-Gómez & Hodgson (2003) place the previous suggestion into the physiological context that HisA and TrpF share a common ancestry (Jürgens *et al.*, 2000; Henn-Sax *et al.*, 2001, 2002).

The crystal structures of PRAI (tTrpF) and ProFARI (tHisA) from *Thermotoga maritima* have been solved (Hennig *et al.*, 1997; Lang *et al.*, 2000) and were shown to have $(\beta\alpha)_8$ or TIM-barrel structures. PriA shares 26 and 16% sequence identity with tHisA and tTrpF, respectively, and hence is also anticipated to have a $(\beta\alpha)_8$-barrel structure. The catalytically important residues from tHisA, Asp7, Asp127 and Thr164 (Jürgens *et al.*, 2000; Henn-Sax *et al.*, 2002), are conserved in PriA of *S. coelicolor*, suggesting that PriA resembles tHisA more closely than tTrpF. TrpF has also been solved from *Escherichia coli*, where it is a bifunctional enzyme comprising of a PRAI domain and an indole-3-glycerol-phosphate synthase (InGPS or TrpC, EC 4.1.1.48; TrpF, EC 5.3.1.24) domain (Priestle *et al.*, 1987).

The evolution of $(\beta\alpha)_8$-barrels has been the subject of much debate (reviewed by Henn-Sax *et al.*, 2001; Gerlt & Raushel, 2003). It is not clear whether this large family of proteins arose by divergent evolution from a common ancestor or by convergent evolution to a stable fold. Functional evidence for a common ancestry includes the directed evolution of the activity of tTrpF from the scaffold of tHisA protein using random mutagenesis and selection *in vivo* (Jürgens *et al.*, 2000). Although the mutant tHisA variant showed only low levels of PRAI activity, albeit at the expense of the

original ProFARI activity, the observation that the exchange of a single residue was sufficient to obtain the new PRAI activity is a remarkable one. Unfortunately, all attempts to elucidate the structure of the mutant tHisA variant with PRAI activity have proved to be unsuccessful (Henn-Sax *et al.*, 2002). As PriA is known to undertake the role of both PRAI and ProFARI in *S. coelicolor* within a physiologically relevant context, its structure may provide further insights into the evolution of this large group of enzymes. In this paper, we report the expression, purification, crystallization and preliminary X-ray diffraction studies of a recombinant version of PriA from *S. coelicolor*, a predicted $(\beta\alpha)_8$-barrel which we anticipate might be an interesting model for studying the evolution of this superfamily of proteins as suggested by its broad substrate specificity.

2. Materials and methods

2.1. Cloning of priA(SCO2050) from *S. coelicolor*

The *priA* gene from *S. coelicolor* PriA (SCO2050) was amplified and cloned by PCR as described previously (Barona-Gómez & Hodgson, 2003). The primers used were GGAATTCCATATGAGCAAGCTCGAACTC and CGGGATCCGGGCGAAACCGAAGGACTC and the PCR product obtained was inserted into the expression vector pET15b (Novagen) using the *Nde*I and *Bam*HI restriction enzymes and T4 DNA ligase (Gibco) to generate the expression plasmid pETpriASc. The construct was confirmed by sequencing and used to transform *E. coli* strain C41(DE3) (Miroux & Walker, 1996) containing the auxiliary plasmid pRIL that encodes rare tRNAs (Stratagene).

2.2. Assay for the PRAI activity of PriA

N′-(5′-phosphoribosyl)anthranilate (PRA) was synthesized *in situ* (Kirschner *et al.*, 1987) and PRA isomerase activity was measured by detecting the production of 1-(*O*-carboxyphenylamino)-1-deoxy-ribulose-5-phosphate at 350 nm. The reaction mixture contained 80 m*M* Tris–HCl pH 8.6, 0.4 m*M* EDTA, 0.1 m*M* anthranilic acid and 0.1 m*M* ribose-5-phosphate (purchased from Sigma). This assay was used to monitor the activity of PriA during the purification steps.

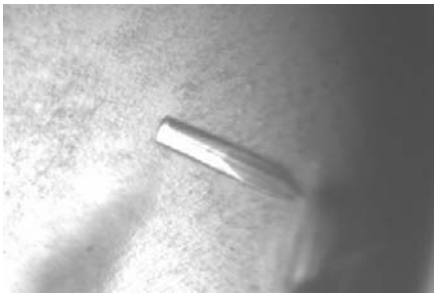

Figure 1
Photograph of a PriA crystal; the largest dimension is 0.3 mm.

2.3. Overexpression and purification of PriA

Overexpressed PriA was purified as a six-His-tagged fusion from the expression plasmid pETpriASc in *E. coli* strain C41(DE3) (pRIL) in Luria–Bertani broth. Soluble protein was obtained after induction with 1 m*M* IPTG at an OD_{600} of between 0.6 and 0.8 and overnight expression at 298 K. The cells from a 0.5 l culture were harvested and resuspended in 25 ml of ice-cold 50 m*M* Tris–HCl pH 8.0, 0.5 m*M* EDTA, 50 m*M* NaCl, 5% glycerol, 0.1 m*M* DTT and 100 µg ml^{-1} lysozyme. The cells were sonicated at 70% power for 4 min at 20 s intervals.

The sonicate was centrifuged at 16 000 rev min^{-1} for 20 min at 277 K and the soluble extract was bound to 3 ml of chelating Sepharose Fast Flow (Amersham Pharmacia) activated with $NiCl_2$. The Sepharose was washed twice with 50 m*M* HEPES pH 8, 1 *M* NaCl, 10 m*M* imidazole and once with 50 m*M* HEPES pH 8, 1 *M* NaCl, 20 m*M* imidazole and PriA was eluted with 50 m*M* HEPES pH 8, 100 m*M* NaCl, 100 m*M* imidazole.

The eluate was concentrated by ultrafiltration and loaded onto a Superdex 75 HR26/60 column (Amersham Pharmacia) at 277 K pre-equilibrated with 50 m*M* Tris–HCl pH 8, 200 m*M* NaCl, 1 m*M* EDTA and 0.5 m*M* DTT. The column flow rate was 2 ml min^{-1} and 5 ml fractions were collected. Fractions containing PriA were identified using the enzyme assay described above. Active fractions were pooled and concentrated to approximately 10 mg ml^{-1} for crystallization trials. The degree of purity of PriA as determined by SDS–PAGE was greater than 95%.

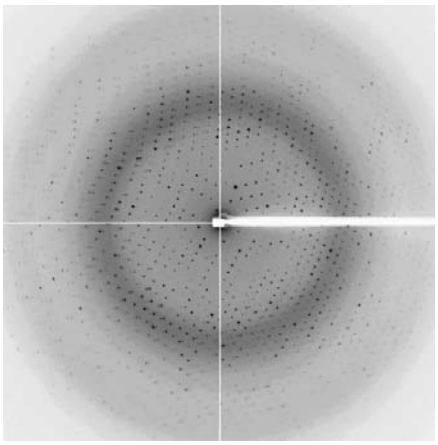

Figure 2
A typical diffraction image collected at beamline ID14-1 at the ESRF using an ADSC Q4 CCD detector. The oscillation range was 1° and the resolution at the edge is 2 Å.

Table 1
Data-collection and processing statistics.

Values in parentheses refer to the highest resolution shell (2.07–2.00 Å).

Synchrotron radiation	ESRF ID14-1
Detector	ADSC Q4 CCD
Wavelength (Å)	0.934
Space group	$P3_{1,2}21$
Unit-cell parameters	
a (Å)	65.1
c (Å)	104.7
Molecules per AU	1
Matthews coefficient (Å^3 Da^{-1})	2.3
Solvent convent (%)	47
Resolution range (Å)	39–2.0
Total observations	135807
Unique reflections	17097
Average $I/\sigma(I)$	21.7 (5.3)
$R_{\rm merge}$ (%)	0.093 (0.389)
Completeness (%)	95.2 (98.6)

2.4. Crystallization

Initial crystallization trials were performed with screens from Molecular Dimensions Ltd (MDL) and Emerald Biostructures Inc. using the hanging-drop vapour-diffusion technique. Needle-shaped crystals were obtained with condition No. 30 from the MDL Structure Screen 1 [0.1 *M* Na HEPES pH 7.5, 2%(*v*/*v*) PEG 400 and 2 *M* ammonium sulfate] using 1 µl of protein at ∼10 mg ml^{-1} mixed with an equal volume of mother liquor. After optimization, crystals grew after 4–5 d at 291 K in mother liquor consisting of 1.50 *M* ammonium sulfate and 100 m*M* sodium citrate pH 4.8 (Fig. 1). The largest crystals obtained were 0.05 × 0.05 × 0.3 mm in size.

2.5. Data collection and processing

Prior to data collection, PriA crystals were cryoprotected by dipping in mother liquor containing 30% ethylene glycol for 2–3 s and were immediately frozen in the N_2 cryostream. Initial diffraction experiments were performed at SRS, Daresbury, but a complete native data set was collected at 100 K on beamline ID14-1 at the ESRF. The beamline operates at a wavelength of 0.934 Å and is equipped with an ADSC Q4 CCD detector. A typical diffraction pattern is shown in Fig. 2. All data were indexed, integrated and scaled with the *HKL* package

(Otwinowski & Minor, 1997) and the data-collection and processing statistics are shown in Table 1. A heavy-atom search and selenomethionine expression of the protein is in progress in order to solve the structure by multiple isomorphous replacement or multiple wavelength anomalous dispersion.

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