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Overexpression, crystallization and preliminary X-ray crystallographic analysis of pyridoxal biosynthesis lyase PdxS from *Pyrococcus horikoshii*

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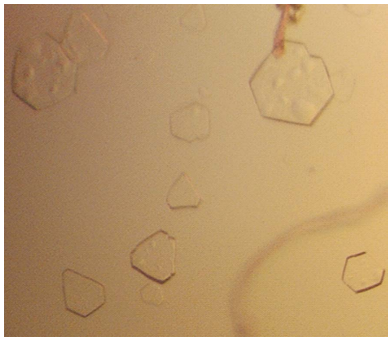
Pyridoxal biosynthesis lyase (PdxS) is an important player in the biosynthesis of pyridoxal 5′-phosphate (PLP), the biologically active form of vitamin B_6. PLP is an important cofactor involved in the metabolic pathway of amine-containing natural products such as amino acids and amino sugars. PdxS catalyzes the condensation of ribulose 5-phosphate (Ru5P), glyceraldehyde 3-phosphate (G3P) and ammonia, while glutamine amidotransferase (PdxT) catalyzes the production of ammonia from glutamine. PdxS and PdxT form a complex, PLP synthase, and widely exist in eubacteria, archaea, fungi and plants. To facilitate further structural comparisons among PdxS proteins, the structural analysis of PdxS from *Pyrococcus horikoshii* encoded by the *Ph1355* gene was initiated. PdxS from *P. horikoshii* was overexpressed in *Escherichia coli* and crystallized at 296 K using 2-methyl-2,4-pentanediol as a precipitant. Crystals of *P. horikoshii* PdxS diffracted to 2.61 Å resolution and belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 59.30, b = 178.56, c = 109.23 Å, $\beta = 102.97^\circ$. The asymmetric unit contained six monomers, with a corresponding V_M of 2.54 Å^3 Da^{-1} and a solvent content of 51.5% by volume.

1. Introduction

Vitamin B_6 is an essential cofactor for various enzymes and is required for maintenance of the nervous and immune systems in animals and humans (Bender, 1989). One of the biologically active forms of vitamin B_6 is pyridoxal 5′-phosphate (PLP), which is essential for many enzyme reactions such as transamination, decarboxylation, racemization and elimination in amino-acid metabolism, DNA biosynthesis and biosynthesis of antibiotic compounds (Eliot & Kirsch, 2004; Mehta & Christen, 2000; Percudani & Peracchi, 2003). There are two pathways for *de novo* PLP biosynthesis and both pathways exist in bacteria, fungi, protozoa and plants, whereas they are missing in mammals (Belitsky, 2004; Dong *et al.*, 2004; Osmani *et al.*, 1999). The first pathway is employed by many eubacteria to produce PLP from erythrose 4-phosphate and 1-deoxy-D-xylulose 5-phosphate (Cane *et al.*, 1999; Laber *et al.*, 1999). In the second pathway, PLP is synthesized from ribose 5-phosphate (R5P) or its isomer ribulose 5-phosphate (Ru5P), dihydroxyacetone phosphate (DHAP) or its isomer glyceraldehyde 3-phosphate (G3P), and ammonia. Pyridoxal biosynthesis lyase (PdxS) and glutamine amidotransferase (PdxT) form a complex, PLP synthase, and are responsible for the second *de novo* PLP-biosynthetic pathway (Zein *et al.*, 2006).

Several crystal structures of PdxS (and homologues such as Pdx1, YaaD and Snz1) and PdxT (and homologues such as Pdx2, YaaE and Sno1) have been reported. The crystal structure of PdxS from *Geobacillus stearothermophilus* shows that PdxS is composed of a cylindrical dodecamer (a dimer of two hexameric rings) of subunits having the TIM-barrel fold (PDB entry 1znn; Zhu *et al.*, 2005). The crystal structures of the *Thermotoga maritima* YaaD–YaaE complex in complex with Ru5P (PDB entry 2iss; Zein *et al.*, 2006) and the *Bacillus subtilis* Pdx1–Pdx2 complex (PDB entry 2nv2; Strohmeier *et al.*, 2006) have been reported. The Pdx1–Pdx2 complex contains 24 subunits, consisting of dodecameric Pdx1 and dodecameric Pdx2. The crystal structures of *Saccharomyces cerevisiae* Snz1 (apo, Snz1–G3P



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complex and Snz1–PLP complex; PDB entries 3o06, 3o07 and 3o05, respectively) have recently been reported (Zhang *et al.*, 2010). The glutaminase PdxT is inactive in the absence of the synthase subunit PdxS (Strohmeier *et al.*, 2006). It was proposed from the structures that an oxyanion hole in the active site of PdxT is triggered by a peptide flip induced by interaction with PdxS (Strohmeier *et al.*, 2006). The ammonia produced by PdxT is thought to be transferred through an internal tunnel in the the PdxS subunit (Strohmeier *et al.*, 2006).

In order to facilitate further structural comparisons among PdxS proteins, we are interested in determining the three-dimensional structure of PdxS from *P. horikoshii* (*Ph1355*), which shares 50–60% amino-acid sequence identity with the structurally characterized PdxS proteins (62% with that from *T. maritima*, 59% with that from *B. subtilis* and 54% with that from *S. cerevisiae*). PdxS from *P. horikoshii* has been overexpressed in *Escherichia coli* and crystallized. Its crystallization conditions and the collection of X-ray crystallographic data are reported here.

2. Materials and methods

2.1. Protein expression and purification

The *Ph1355* gene encoding PdxS of *P. horikoshii* was amplified from the genomic DNA by the polymerase chain reaction. The forward and reverse oligonucleotide primers were 5′-G GAA TTC **CAT ATG** GAC AAG TTG AAA ATC ATT ATG GAA AAG-3′ and 5′-CCG CCG **CTC GAG** TTA GAT ACC TCT CTC CTC CAT TCT A-3′, respectively. The bases in bold represent *Nde*I and *Xho*I restriction-enzyme cleavage sites. The amplified DNA was digested with *Nde*I and *Xho*I and was then inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a(+) (Novagen). The plasmid was transformed into *E. coli* strain Rosetta2 (DE3) pLysS cells for protein expression. The cells were grown at 310 K to an OD_{600} of 0.5 in Terrific Broth medium containing 50 µg ml^{-1} ampicillin and protein expression was induced by 0.5 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG). Cell growth continued at 291 K for 48 h after IPTG induction and the cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer *A* (50 m*M* Tris–HCl pH 7.9, 200 m*M* NaCl) containing 1 m*M* phenylmethylsulfonyl fluoride and was homogenized with an ultrasonic processor and then heated for 10 min at 343 K. The crude cell extract was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 1 h at 277 K. The supernatant was subjected to ion-exchange chromatography on a HiLoad 26/10 Q Sepharose HP column (GE Healthcare) which had previously been equilibrated with buffer *A* and the protein was eluted with a linear gradient of 0–1.0 *M* NaCl in buffer *A*. Further purification was performed by gel filtration on a HiLoad 16/60 Superdex 200 prep-grade column (GE Healthcare) which was previously equilibrated with buffer *A*. The homogeneity of the purified protein was assessed by polyacrylamide gel electrophoresis in the presence of 0.1%(*w*/*v*) sodium dodecyl sulfate (Laemmli, 1970; Fig. 1). The protein solution was concentrated to about 18 mg ml^{-1} using an YM10 ultrafiltration membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated molar extinction coefficient of 19 940 M^{-1} cm^{-1} (SwissProt; http://www.expasy.org/).

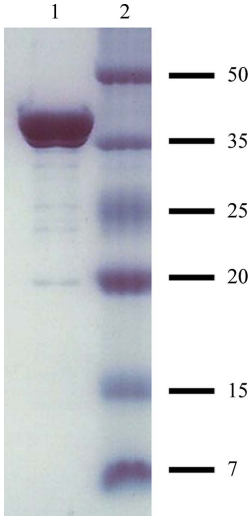

Figure 1
Coomassie-stained gel of PdxS from *P. horikoshii* isolated by size-exclusion chromatography. Lane 1, PdxS protein; lane 2, size markers (labelled in kDa).

2.2. Crystallization, dynamic light scattering and X-ray data collection

Crystallization experiments were carried out using the hanging-drop vapour-diffusion method at 296 K using 24-well tissue-culture plates (Hampton Research). A hanging drop was prepared on a siliconized cover slip by mixing equal volumes (2 µl each) of protein solution and reservoir solution. The hanging drop was placed over 0.5 ml reservoir solution. Initial crystallization conditions were established using screening kits from Hampton Research (Crystal Screen, Crystal Screen 2 and MembFac) and from Emerald BioStructures (Wizard I and II). Dynamic light-scattering experiments were performed using a DynaPro-801 instrument from Wyatt (Santa Barbara, California, USA). The data were measured at 297 K with the protein at 1 mg ml^{-1} in buffer *A*. Crystals of PdxS from *P. horikoshii* obtained using 2-methyl-2,4-pentanediol as a precipitant were further optimized using a detergent screening kit (Hampton Research).

Crystals were flash-cooled in a liquid-nitrogen stream employing 35%(*v*/*v*) 2-methyl-2,4-pentanediol as a cryoprotectant. X-ray diffraction data for *P. horikoshii* PdxS were collected at 100 K at the BL-4A experimental station of Pohang Light Source, Pohang, Republic of Korea with an ADSC Quantum 270 CCD detector system (Area Detector Systems Corporation, Poway, California,

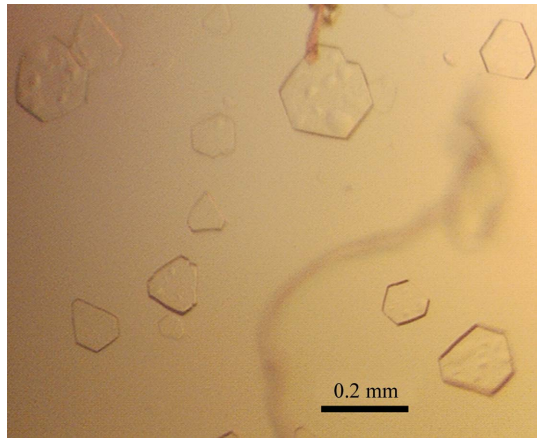

Figure 2
Crystals of pyridoxal biosynthesis lyase PdxS from *P. horikoshii*. Approximate dimensions are 0.18 × 0.18 × 0.08 mm.

Table 1
Data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

X-ray wavelength (Å)	1.0629
Detector	ADSC Quantum 270 CCD detector
No. of crystals	1
Crystal-to-detector distance (mm)	240
Rotation range per frame (°)	1
Total rotation range (°)	300
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 59.30, b = 178.56, c = 109.23, β = 102.97
Resolution range (Å)	50–2.61 (2.70–2.61)
Total/unique reflections	420103/66140
Completeness (%)	100 (100)
Mean $I/\sigma(I)$	36.2 (5.4)
$R_{\rm merge}$† (%)	8.0 (37.3)
Mosaicity (°)	0.3
Multiplicity	6.4 (6.3)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

USA). The wavelength of the synchrotron X-rays was 1.0629 Å and a 0.3 mm collimator was used. The crystal was rotated through a total of 300° with 1.0° oscillation range per frame. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

P. horikoshii PdxS in its intact form was overexpressed in soluble form with a yield of ∼15 mg homogeneous protein per litre of culture. The best crystals were produced by mixing equal volumes (1.8 µl each) of protein solution (18 mg ml^{-1} in 50 m*M* Tris–HCl pH 7.9, 200 m*M* NaCl) and reservoir solution [100 m*M* imidazole pH 8.0, 35%(*v*/*v*) 2-methyl-2,4-pentanediol, 200 m*M* $MgCl_2$] and 0.4 µl 104 m*M* *N*,*N*-dimethyldecylamine-β-oxide. The crystals grew to maximum dimensions of 0.18 × 0.18 × 0.08 mm within two weeks (Fig. 2). A set of X-ray diffraction data was collected to 2.61 Å resolution at 100 K. A total of 420 103 measured reflections were merged into 66 140 unique reflections, giving an $R_{\rm merge}$ of 8.0% and a completeness of 100%. The space group was determined to be $P2_1$ on the basis of systematic absences and symmetry of diffraction intensities. The unit-cell parameters are a = 59.30, b = 178.56, c = 109.23 Å, β = 102.97°. Table 1 summarizes the statistics of data collection. The molecular mass of the recombinant PdxS was estimated to be ∼200 kDa by dynamic light-scattering analysis, indicating that the enzyme exists as a hexamer in solution (calculated monomer mass of 37 014 Da). If it is assumed that a hexameric molecule is present in the crystallographic asymmetric unit, the crystal volume per protein mass ($V_{\rm M}$) is 2.54 Å^3 Da^{-1} and the solvent content is 51.5% (Matthews, 1968).

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