Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Purification, crystallization and preliminary crystallographic analysis of a 6-pyruvoyltetrahydropterin synthase homologue from *Esherichia coli*

Kyung Hye Seo,[a,b,c] Supangat,[a,b,c] Hye Lim Kim,[d] Young Shik Park,[d] Che Ok Jeon[a,b,c] and Kon Ho Lee[a,b,c]*

[a]Division of Applied Life Science, Gyeongsang National University, Jinju 660-711, Republic of Korea, [b]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-711, Republic of Korea, [c]Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-711, Republic of Korea, and [d]Mitochondrial Research Group, School of Biotechnology and Biomedical Science, Inje University, Kimhae 621-749, Republic of Korea

Correspondence e-mail: lkh@gsnu.ac.kr

Received 4 December 2007
Accepted 8 January 2008

6-Pyruvoyltetrahydropterin synthase from *E. coli* (ePTPS) has been crystallized using the hanging-drop vapour-diffusion method. Hexagonal- and rectangular-shaped crystals were obtained. Diffraction data were collected from the hexagonal and rectangular crystals to 3.0 and 2.3 Å resolution, respectively. The hexagonal plate-shaped crystals belonged to space group *P*321, with unit-cell parameters $a = b = 112.59$, $c = 68.82$ Å, and contained two molecules in the asymmetric unit. The rectangular crystals belonged to space group *I*222, with unit-cell parameters $a = 112.76$, $b = 117.66$, $c = 153.57$ Å, and contained six molecules in the asymmetric unit. The structure of ePTPS in both crystal forms has been determined by molecular replacement.

1. Introduction

6-Pyruvoyltetrahydropterin synthase (PTPS; EC 4.2.3.12) is the second enzyme in the biosynthesis of tetrahydrobiopterin (BH4) from GTP; it catalyzes the conversion of dihydroneopterin triphosphate (H2-NTP) to 6-pyruvoyltetrahydropterin (PPH4) (Thöny *et al.*, 2000; Fig. 1*a*). BH4 is a well known essential cofactor for aromatic amino-acid hydroxylases and nitric oxide synthases in higher animals. However, BH4 is not common in bacteria, although its glycosidic forms have been found in particular species such as *Cyanobacteria* (Chung *et al.*, 2000) and anaerobic photosynthetic *Chlorobium* species (Cho *et al.*, 1998).

Interestingly, PTPS homologues have been found in the genome sequences of many bacteria that are known not to produce BH4 or its glycosides. A bacterial PTPS homologue (bacterial PTPS-I; bPTPS-I) from *Synechococcus* sp. PCC 6803 was found to possess the animal PTPS activity of converting H2-NTP to PPH4 (Lee *et al.*, 1999). However, subsequent work with the recombinant *Escherichia coli* and *Synechococcus* sp. PCC 6803 enzymes revealed that bPTPS-Is have another peculiar catalytic function in which they cleave the C6 side chain of sepiapterin to generate dihydropterin *in vitro*; this activity was barely detectable in human PTPS (Woo *et al.*, 2002; Fig. 1*b*). This catalytic function does not suggest any comparable

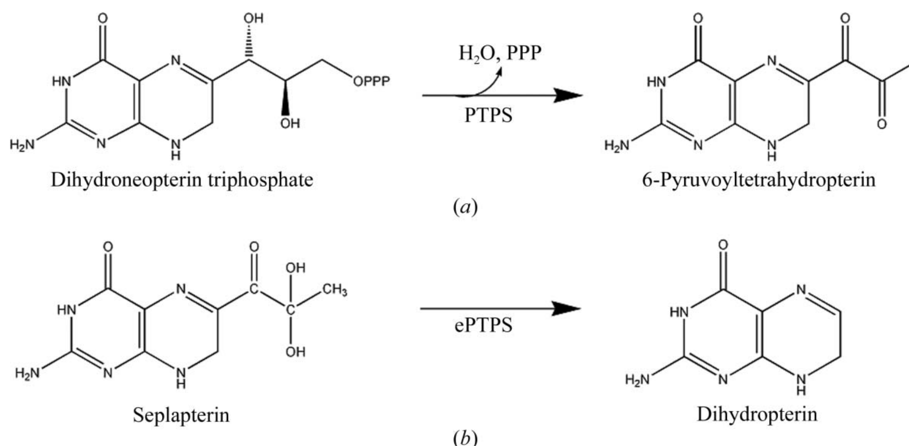
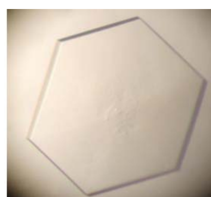


Figure 1
The enzymatic reaction catalyzed by 6-pyruvoyltetrahydropterin synthase from (*a*) human and rat and (*b*) *E. coli*.



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physiological role of bPTPS-Is in bacteria because sepiapterin is not an indigenous metabolite. In addition, another bacterial PTPS homologue (bacterial PTPS-II; bPTPS-II) consisting of two PTPS domains organized in tandem was found to have genuine PTPS activity in *Synechococcus* sp. PCC 7942 (Kong *et al.*, 2006). Therefore, it seems that bPTPS-Is perform certain as yet undetermined functions in bacteria.

In this paper, we report the expression, purification, crystallization and preliminary crystallographic analysis of *E. coli* PTPS (ePTPS). Knowledge of the three-dimensional structure of ePTPS will provide a deeper understanding of its extraordinary enzyme activity in comparison with animal enzymes. ePTPS (121 residues) shares 27% sequence identity to human PTPS (145 residues) and 26% to rat PTPS (144 residues). The N-terminal region of ePTPS has no sequence similarities to those of human and rat PTPS and contains none of the conserved active-site residues such as Cys42 and His23 for the conversion of H2-NTP to PPH4. ePTPS carries a Zn^{2+} ion for the reaction as found in higher animals, but it exists as a trimer in solution in contrast to the hexameric animal enzymes. The peculiar enzymatic activity of ePTPS may be a consequence of these differences.

2. Experimental

2.1. Protein expression and purification

The ePTPS gene was cloned into pET-28b (Novagen, Madison, Wisconsin, USA) expression plasmid to produce recombinant ePTPS with a hexahistidine tag and a thrombin cleavage site at the N-terminus (MGSSHHHHHHSSGLVPRGSH). The plasmid was transformed into *E. coli* strain BL21(DE3) for protein expression. 100 ml aliquots of an overnight culture were seeded into 1000 ml fresh LB (Luria–Bertani) medium (10 g Bacto tryptone, 5 g yeast extract, 10 g NaCl per litre of solution) containing kanamycin (30 µg ml^{-1}) and allowed to grow to an OD_{600} of 0.6 at 300 K. Protein expression was induced for 4 h with 0.4 m*M* isopropyl β-D-1-thiogalactopyranoside and cells were harvested by centrifugation (6 min, 277 K, 6000 rev min^{-1}). The harvested cells were washed with phosphate-buffered saline (PBS; 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 per litre of solution, pH 7.4), resuspended in lysis buffer (50 m*M* sodium phosphate pH 8.0, 0.5 *M* NaCl, 5 m*M* imidazole) and disrupted by sonication. After centrifugation (1 h, 12 000 rev min^{-1}) at 277 K, the clear supernatant was filtered (Qualitative filter paper, Advantec) and applied onto a column of nickel–NTA beads (Qiagen, Hilden, Germany) pre-equilibrated with lysis buffer. The column was first washed with ten column volumes of lysis buffer and then with 20 column volumes of 50 m*M* Tris–HCl pH 8.0, 0.5 *M* NaCl, 30 m*M* imidazole. The recombinant ePTPS was eluted with 50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 300 m*M* imidazole. Fractions containing ePTPS were pooled, concentrated and exchanged into 50 m*M* Tris–HCl pH 8.0 by ultrafiltration (Centriprep YM-30, Millipore Corporation, Bedford, Massachusetts, USA). The ePTPS was further purified by anion-exchange chromatography on a Resource 15Q column (GE Healthcare, Piscataway, New Jersey, USA). The protein was eluted using a salt gradient and separated at ∼0.3 *M* NaCl at pH 8.0. The fractions containing ePTPS were concentrated by ultrafiltration (Centriprep YM-30, Millipore Corporation, Bedford, Massachusetts, USA). The ePTPS was finally purified by gel-filtration chromatography with a Superdex 200 column (GE Healthcare, Piscataway, New Jersey, USA) in PBS or in 20 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl. The fractions containing ePTPS were then exchanged into PBS or 20 m*M* Tris–HCl pH 8.0 and concentrated to a final concentration of 10 mg ml^{-1} by ultrafiltration (Microcon YM-30, Millipore Corporation, Bedford, Massachusetts, USA). The protein purity was examined by SDS–PAGE and native PAGE. The protein concentration was determined using the Bradford method. The N-terminal His tag was not removed for crystallization.

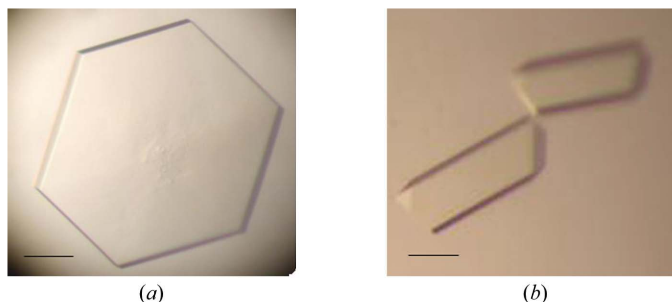

Figure 2
Crystals of 6-pyruvoyltetrahydropterin from *E. coli*. The two crystal forms have approximate dimensions of (*a*) 0.7 × 0.7 × 0.3 mm and (*b*) 0.6 × 0.3 × 0.2 mm, respectively. The scale bar in the pictures corresponds to 0.2 mm.

2.2. Crystallization and data collection

Crystallization were initially carried out with Crystal Screens I and II and Index Screen (Hampton Research, California, USA) and Wizard Screens I, II, Cryo I and II (Emerald BioStructures, Bainbridge Island, Washington, USA) using the hanging-drop vapour-diffusion method at 291 K. The drops, which contained equal volumes (1 µl) of protein solution (10 mg ml^{-1}) and reservoir solution, were equilibrated against 0.5 ml reservoir solution. Rod-shaped microcrystals (crystal form I) were produced with Index Screen solution No. 59 (0.02 *M* $MgCl_2$, 0.1 *M* HEPES pH 7.5, 22% polyacrylic acid 5100 sodium salt) using protein in PBS. Further screenings to find optimal crystallization conditions for crystal growth were accomplished by varying the salt and precipitant concentrations and the volume of the drop and using Index Screen solutions as additives with the hanging-drop vapour-diffusion method. The best large crystals were obtained at 291 K in a drop containing 3 µl of 10 mg ml^{-1} protein solution in PBS and 4 µl of a mixture of 4 µl reservoir solution (0.02 *M* $MgCl_2$, 0.1 *M* HEPES pH 7.5, 22% polyacrylic acid 5100 sodium salt) and 1 µl Index Screen solution No. 9 (0.1 *M* bis-Tris pH 5.5, 3 *M* NaCl) as an additive. Crystals grew to maximum dimensions of 0.7 × 0.7 × 0.3 mm (Fig. 2*a*). Using protein in 20 m*M* Tris–HCl pH 8.0, needle-shaped microcrystals (crystal form II) were obtained using Index Screen solution No. 88 (0.2 *M* triammonium citrate pH 7.0, 20% PEG 3350). The best crystals of form II were produced at 291 K in a drop containing 3 µl of 10 mg ml^{-1} protein solution and 3 µl of a mixture of 4 µl reservoir solution (0.16 *M* triammonium citrate pH 7.0, 16% PEG 3350) and 1 µl additive (1 *M* guanidine hydrochloride); crystals grew to maximum dimensions of 0.6 × 0.3 × 0.2 mm (Fig. 2*b*). Crystals were flash-frozen in liquid nitrogen for data collection after soaking for 30 min in reservoir solution with 18% ethylene glycol for crystal form I and in reservoir solution with 20% ethylene glycol for crystal form II.

X-ray diffraction data for ePTPS crystal form I were collected to 3.0 Å resolution from a single crystal using X-rays of wavelength 1.123 Å and a Bruker CCD detector at station 6B of the Pohang Accelerator Laboratory, Pohang, Republic of Korea. The crystal-to-detector distance was set to 280 mm; 1° oscillation and 30 s exposure were used per image. Data were collected from ePTPS crystal form II at beamline 4A of the Pohang Accelerator Laboratory, Pohang, Republic of Korea using X-rays of wavelength 1.000 Å. Exposure

Table 1
Data statistics for ePTPS crystals.

Values in parentheses are for the highest resolution shell.

Space group	*P*321	*I*222
Unit-cell parameters (Å)	$a = b = 112.59$, $c = 68.82$	$a = 112.76$, $b = 117.66$, $c = 153.57$
No. of molecules in ASU	2	6
V_M (Å^3 Da^{-1})	3.51	2.34
Wavelength (Å)	1.123	1.000
Resolution† (Å)	50–3.0 (3.11–3.00)	50–2.34 (2.42–2.34)
Unique reflections	10571 (726)	43223 (4508)
Completeness (%)	94.8 (65.8)	99.9 (98.5)
R_{merge}‡ (%)	23.1 (79.4)	12.2 (94.4)
Redundancy	9.0	12.2
$\langle I/\sigma(I) \rangle$	7.2	10.4
Solvent content (%)	60.95	57.61

† The resolution range used for molecular replacement was 15.0–3.5 Å for *P*321 and 15.0–3.0 Å for *I*222. ‡ $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \overline{I(hkl)}| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th intensity measurement of reflection *hkl*, including symmetry-related reflections, and $\overline{I(hkl)}$ is its average.

times were 15 s for 1° oscillations at 230 mm crystal-to-detector distance. All diffraction images were indexed, integrated and scaled using the *HKL*-2000 program suite (Otwinowski & Minor, 1997). Data statistics are shown in Table 1.

3. Results

We have established the expression, purification and crystallization of ePTPS. A 3.0 Å resolution data set was collected from crystal form I and processed. The space group was found to be the hexagonal space group *P*321, with unit-cell parameters $a = b = 112.59$, $c = 68.82$ Å (Table 1). The molecular weight of the protein was estimated to be about 45 kDa from gel-filtration chromatography and that of the monomer of ePTPS to be approximately 15 kDa from SDS–PAGE, which is similar to the theoretical molecular weight of 15.3 kDa calculated from the sequence, suggesting that the protein exists as a trimer in solution. The structure of ePTPS in space group *P*321 could be determined by molecular replacement using the program *AMoRe* (Navaza & Vernoslova, 1995) with *Pseudomonas aeruginosa* PTPS (PDB code 2oba; T. E. McGrath, G. Kisselman, K. Battaile, V. Romanov, J. Wu-Brown, J. Guthrie, C. Virag, K. Mansoury, A. M. Edwards, E. F. Pai & N. Y. Chirgadze, unpublished work), with which it shares 69% amino-acid sequence identity, as a search model (correlation coefficient = 59.3, *R* factor = 50.4% after translation search). The structure could not be solved using the rat PTPS structure (PDB code 1gtq; Nar *et al.*, 1994) as a model. This suggests that the three-dimensional structures of bacterial PTPS-I differ from the animal enzyme structures. There were two molecules in the asymmetric unit, with a solvent content of 60.95% and a V_M value of 3.51 Å^3 Da^{-1} (Matthews, 1968; Table 1). In the crystal, ePTPS forms a hexamer constructed by two closely interacting trimers generated by the crystallographic symmetry mates of each molecule in the asymmetric unit (Fig. 3).

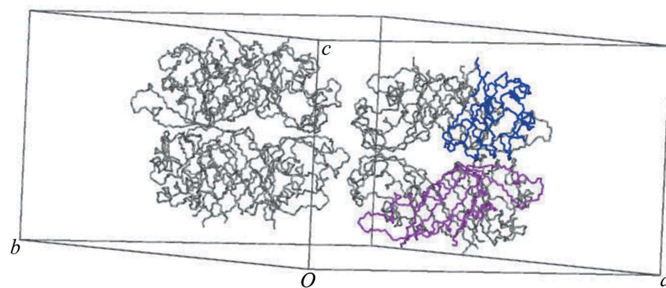

Figure 3
ePTPS cystal packing in space group *P*321. A hexamer of ePTPS was formed by two trimers that were generated from the crystallographic symmetry mates of the two monomers shown in blue and magenta.

A 2.3 Å resolution data set was collected from crystal form II and processed; the crystal had a body-centred orthorhombic lattice with ambiguity in the space group between *I*222 and $I2_12_12_1$ and with unit-cell parameters $a = 112.76$, $b = 117.66$, $c = 153.57$ Å. The space group of crystal form II was determined to be *I*222 by molecular replacement using the program *AMoRe* (Navaza & Vernoslova, 1995) with the model of ePTPS determined in space group *P*321 as the search model (correlation coefficient = 61.5, *R* factor = 52.3% after translation search). There were six molecules in the asymmetric unit, forming two trimers, with a solvent content of 57.61% and a V_M value of 2.34 Å^3 Da^{-1} (Table 1). In this crystal, one hexamer formed by two closely interacting trimers was present in the asymmetric unit. Detailed discussion of the refined structures of both crystal forms will be published elsewhere.

We thank the staff at beamlines 6B, 6C and 4A of Pohang Accelerator Laboratory for help with data collection. This work was supported by Korea Research Foundation grant KRF-2006-521-C00153 (KHL), by the BK21 program (KHS and S), grant R15-2003-002-01001-0 to the EB-NCRC (KHL) and the Inje Academic Research Fund 2006 (YSP).

References

Cho, S. H., Na, J. U., Youn, H., Hwang, C. S., Lee, C. H. & Kang, S. O. (1998). *Biochim. Biophys. Acta*, **1379**, 53–60.
Chung, H. J., Kim, Y. A., Kim, Y. J., Choi, Y. K., Hwang, Y. K. & Park, Y. S. (2000). *Biochim. Biophys. Acta*, **1524**, 183–188.
Kong, J. S., Kang, J. Y., Kim, H. L., Kwon, O. S., Lee, K. H. & Park, Y. S. (2006). *FEBS Lett.* **580**, 900–904.
Lee, S. W., Lee, H. W., Chung, H. J., Kim, Y. A., Kim, Y. J., Hahn, Y., Chung, J. H. & Park, Y. S. (1999). *FEMS Microbiol. Lett.* **176**, 169–176.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nar, H., Huber, R., Heizmann, C. W., Thöny, B. & Bürgisser, D. (1994). *EMBO J.* **13**, 1255–1262.
Navaza, J. & Vernoslova, E. (1995). *Acta Cryst.* A**51**, 445–449.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Thöny, B., Auerbach, G. & Blau, N. (2000). *Biochem. J.* **347**, 1–16.
Woo, H. J., Kang, J. Y., Choi, Y. K. & Park, Y. S. (2002). *Appl. Environ. Microbiol.* **68**, 3138–3140.