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Crystallization and preliminary X-ray diffraction analysis of a novel type of phosphoserine phosphatase from *Hydrogenobacter thermophilus* TK-6

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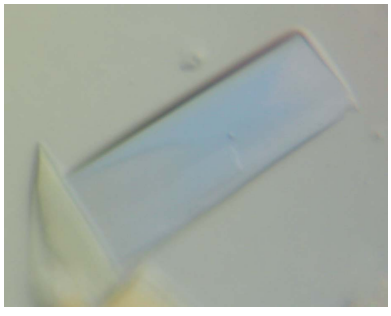

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Two novel-type phosphoserine phosphatases (PSPs) with unique substrate specificity from the thermophilic and hydrogen-oxidizing bacterium *Hydrogenobacter thermophilus* TK-6 have previously been identified. Here, one of the PSPs (iPSP1) was heterologously expressed in *Escherichia coli*, purified and crystallized. Diffraction-quality crystals were obtained by the sitting-drop vapour-diffusion method using PEG 4000 as the precipitant. Two diffraction data sets with resolution ranges of 45.0–2.50 and 45.0–1.50 Å were collected from a single crystal and were merged to give a highly complete data set. The space group of the crystal was identified as primitive orthorhombic $P2_12_12_1$, with unit-cell parameters a = 49.8, b = 73.6, c = 124.3 Å. The calculated Matthews coefficient (V_M = 2.32 Å^3 Da^{-1}) indicated that the crystal contained one iPSP1 complex per asymmetric unit.

1. Introduction

Two novel-type phosphoserine phosphatases (PSPs), iPSP1 and iPSP2, have recently been purified, identified and characterized from *Hydrogenobacter thermophilus* TK-6, an obligately chemolithoautotrophic and thermophilic bacterium that lacks typical PSP genes (Chiba *et al.*, 2012). The purified novel-type PSPs displayed metal-ion-independent activity, whereas typical PSPs, which belong to the haloacid dehalogenase-like hydrolase superfamily, exhibit Mg^{2+}-dependent activity. Both iPSP1 and iPSP2 had high substrate specificity for L-phosphoserine and were strongly suggested to function as PSPs *in vivo* by producing L-serine from L-phosphoserine. However, the structural basis for such strict substrate specificity in iPSPs has not yet been determined.

iPSP1 is a homodimer of PspA subunits, while iPSP2 is a heterodimer of PspA and PspB subunits. PspA and PspB share 35% sequence identity and belong to the histidine phosphatase superfamily. Although PspA and PspB show significant sequence similarity to cofactor-dependent phosphoglycerate mutase (dPGM) from *Escherichia coli* (22 and 18% identity, respectively), they possess neither mutase activity nor the conserved residues important for such activity (Chiba *et al.*, 2012). Similar dPGM-like proteins have been identified in several organisms, although their physiological roles remain unclear (Rigden *et al.*, 2001; Bourgis *et al.*, 2005; Hills *et al.*, 2011).

PhoE, a dPGM-like protein from *Bacillus stearothermophilus*, is a homodimeric phosphatase with broad substrate specificity, but has highest activity towards 3-phosphoglyceric acid and α-naphthyl-phosphate (Rigden *et al.*, 2001). Our previous study revealed that iPSP1, iPSP2 and PhoE exhibit different substrate specificities from one another (Chiba *et al.*, 2012), which suggests differing substrate specificities among the PspA and PspB subunits of iPSP1/2 and the protomer of PhoE. PspA has strict substrate specificity for L-phosphoserine, whereas PspB appears to have lower phosphatase activity towards L-phosphoserine and higher activity towards *para*-nitrophenylphosphate (Chiba *et al.*, 2012). We anticipated that determination of the crystal structure of iPSP1 or iPSP2 and its comparison with that of PhoE (Rigden *et al.*, 2002, 2003) would provide valuable information to determine the basis of the differing substrate specificities in dPGM-like phosphatases.

Table 1
Summary of data-collection statistics for the iPSP1 crystal.

Values in parentheses are for the highest resolution shell.

X-ray source	BL-32XU, SPring-8
Wavelength (Å)	1.0000
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 49.8, b = 73.6, c = 124.3
Predicted solvent content (%)	47.0
Resolution range (Å)	45.0–1.50 (1.54–1.50)
No. of observed reflections	630969 (32454)
No. of unique reflections	73612 (5300)
Data completeness (%)	99.9 (99.0)
$R_{\rm merge}$†	0.091 (0.587)
Multiplicity	8.57 (6.12)
$\langle I/\sigma(I)\rangle$	14.79 (3.28)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

Here, we describe the overexpression, purification, crystallization and preliminary X-ray diffraction analysis of iPSP1.

2. Materials and methods

2.1. Overproduction and purification of iPSP1

The gene encoding the iPSP1 subunit PspA derived from *H. thermophilus* (HTH0103; YP_003431771) was overproduced in *E. coli* and purified as described previously (Chiba *et al.*, 2012). Briefly, the PCR-amplified gene was cloned into the multiple cloning site 1 of pCDFDuet-1 vector (Novagen) using the *Nco*I and *Pst*I sites. PspA lacking any tags was then overexpressed in *E. coli* BL21-CodonPlus(DE3)-RIL cells grown aerobically in Luria–Bertani medium at 310 K. Harvested cells were resuspended in 20 m*M* Tris–HCl pH 8.0 and 1 m*M* EDTA and disrupted by sonication on ice. The homogenates were heat-treated at 353 K for 10 min and centrifuged at 100 000*g* for 1 h and the resulting supernatants were subjected to purification using Butyl-Toyopearl (22 mm × 15 cm; Tosoh) and Mono Q HR 5/5 (1 ml bed volume; GE Healthcare) columns. The purity of iPSP1 was assessed by gel-filtration chromatography using a Superdex 75 HR 10/30 column (GE Healthcare) and 12% SDS–PAGE. The apparent molecular mass of the heterologously expressed PspA subunit of iPSP1 on SDS–PAGE was the same as that of the natively purified subunit (24.3 kDa) and was consistent with the molecular mass calculated from its amino-acid sequence (24.6 kDa; Chiba *et al.*, 2012). A total of ∼3 mg purified iPSP1, the homodimer of the PspA subunit, was obtained from a 1 l *E. coli* culture. Purified iPSP1 was concentrated and desalted to approximately 10 mg ml^{-1} using a 20 ml Vivaspin concentrator (10 kDa cutoff; Vivascience) and 5 m*M* Tris–HCl pH 8.0. Protein concentrations were measured using the Bradford assay kit (Bio-Rad) with bovine serum albumin as a standard.

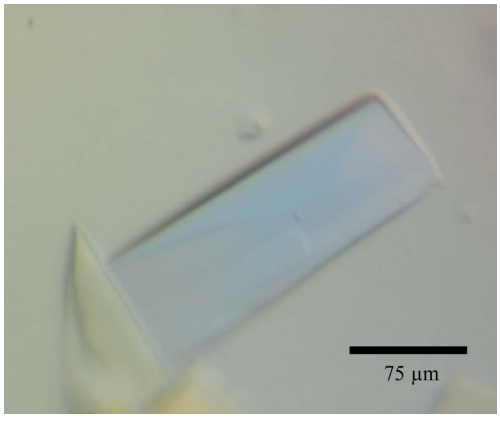

Figure 1
Crystals of iPSP1.

2.2. Crystallization, data collection and preliminary X-ray analysis

Crystallization experiments were performed using commercially available kits, namely Crystal Screen HT, Index HT (Hampton Research) and Wizard Screens I and II (Emerald BioSystems), at 293 K in 96-well Intelli-Plates (Art Robbins). All crystallization droplets were set up manually as follows. A sitting drop was prepared by mixing 0.75 µl protein solution and 0.75 µl reservoir solution and was equilibrated against 50 µl reservoir solution. The protein solution contained ∼10 mg ml^{-1} protein, 5 m*M* Tris–HCl pH 8.0 and less than 0.05 m*M* NaCl. The crystals obtained using a reservoir solution consisting of 100 m*M* HEPES–NaOH pH 7.5, 10%(*v*/*v*) 2-propanol, 20%(*w*/*v*) polyethylene glycol 4000 were used for data collection. The crystals were cryoprotected in crystallization solution supplemented with 25%(*v*/*v*) ethylene glycol, picked up using a mounting loop and cooled in a cold nitrogen-gas stream. Low- and high-resolution diffraction data sets consisting of 180 images each were collected using a MAR225HE detector on beamline BL-32XU at SPring-8, Hyogo, Japan with the following conditions: 1.0000 Å wavelength, 1.0° oscillation angle, an exposure time of 1 s per image and crystal-to-detector distances of 260.0 and 140.0 mm, respectively. Indexing, integration, scaling and merging of the diffraction data were performed with the program *XDS* (Kabsch, 2010).

3. Results and discussion

Plate-shaped crystals of iPSP1 formed in approximately one week using a reservoir composition of 100 m*M* HEPES–NaOH pH 7.5, 10%(*v*/*v*) 2-propanol, 20%(*w*/*v*) polyethylene glycol 4000. The crystals grew to final dimensions of 0.15 × 0.075 × 0.01 mm at 293 K (Fig. 1) and diffracted X-rays to 1.5 Å resolution on beamline BL-32XU at SPring-8 (Fig. 2).

The data-collection statistics for the iPSP1 crystals are summarized in Table 1. The calculated Matthews coefficient ($V_{\rm M}$ = 2.32 Å^3 Da^{-1};

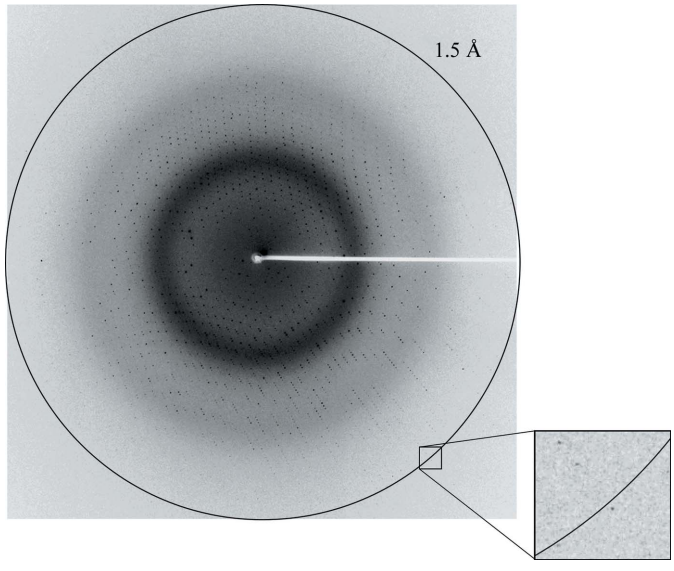

Figure 2
A diffraction image of iPSP1. The circle corresponds to 1.5 Å resolution. The inset represents a magnified view of the area indicated by the square.

Matthews, 1968) indicates that the asymmetric unit contained two PspA protomers, with a solvent content of 47.0%. Our previous gel-filtration data suggested that iPSP1 is a homodimer of PspA subunits (Chiba *et al.*, 2012), which implies that one asymmetric unit contains a complete iPSP1 enzyme. Structural determination of iPSP1 is currently under way using the molecular-replacement method with the atomic coordinates of PhoE from *B. stearothermophilus* (25% sequence identity; PDB entry 1h2e; Rigden *et al.*, 2003) as a search model.

The synchrotron-radiation experiments were performed on beamline BL-32XU at SPring-8, Hyogo, Japan with the approval of the Targeted Proteins Research Program (TPRP) Office at RIKEN/SPring-8. This work was partly supported by the TPRP of the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in-Aid for Scientific Research (A) (21248010) from the Japan Society for the Promotion of Science (JSPS) and a Grant-in-Aid for JSPS fellows (23-3030).

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