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

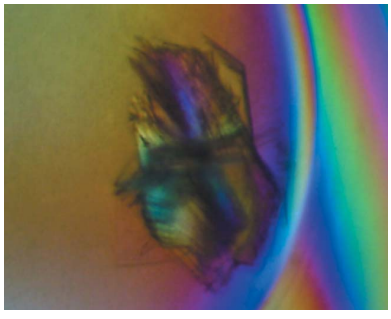
Crystallization and preliminary X-ray crystallographic analysis of a novel histidinol-phosphate phosphatase from *Thermococcus onnurineus* NA1

Ha Il Jung,[a,b] Hyun Sook Lee,[a] Young Jun An,[a,b] Yona Cho,[a] Jung-Hyun Lee,[a] Sung Gyun Kang[a] and Sun-Shin Cha[a]*

[a]Marine and Extreme Genome Research Center, Korea Ocean Research and Development Institute, Ansan 426-744, Republic of Korea, and [b]Department of Biological Sciences, Myongji University, Yongin 449-728, Republic of Korea

Correspondence e-mail: chajung@kordi.re.kr

Received 26 February 2009
Accepted 24 March 2009



The TON_0887 gene product from *Thermococcus onnurineus* NA1 is a 240-residue protein that has histidinol-phosphate phosphatase (HolPase) activity. According to analysis of its primary structure, the TON_0887 gene product is a monofunctional HolPase that belongs to the DDDD superfamily. This contrasts with the generally accepted classification that bifunctional HolPases belong to the DDDD superfamily. The TON_0887 gene product was purified and crystallized at 295 K. A 2.2 Å resolution data set was collected using synchrotron radiation. The TON-HolPase crystals belonged to space group $P222_1$, with unit-cell parameters $a = 40.88$, $b = 46.89$, $c = 148.03$ Å. Assuming the presence of one molecule in the asymmetric unit, the solvent content was estimated to be about 48.3%.

1. Introduction

Histidinol-phosphate phosphatase (HolPase; EC 3.1.3.15) catalyzes the dephosphorylation of histidinol-phosphate to histidinol, the eighth step in the histidine-biosynthetic pathway, in a metal-dependent manner. HolPases can be classified into two types (Brilli & Fani, 2004; Chiariotti *et al.*, 1986; le Coq *et al.*, 1999): the bifunctional HolPases found in proteobacteria such as *Escherichia coli* and the monofunctional HolPases found in archaea, eukarya and most bacteria. Bifunctional HolPases are composed of an N-terminal HolPase domain and a C-terminal domain with imidazoleglycerol-phosphate dehydratase activity (Rangarajan *et al.*, 2006). The N-terminal HolPase domain in this type of HolPase belongs to the DDDD superfamily, where DDDD refers to the four invariant aspartate residues in the sequence (Thaller *et al.*, 1998). A monofunctional HolPase consists of a single domain and belongs to the polymerase and histidinol phosphatase (PHP) superfamily, which is characterized by four motifs with conserved histidine residues (le Coq *et al.*, 1999).

It has been reported that the TON_0887 gene product of *Thermococcus onnurineus* NA1, the full genome sequence of which has recently been published (Lee, Kang *et al.*, 2008), exhibits histidinol-phosphate phosphatase activity that is enhanced in the presence of divalent cations such as Mg^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+} or Cu^{2+} (Lee, Cho *et al.*, 2008). The TON_0887 gene product (TON-HolPase) belongs to the DDDD superfamily as it contains three conserved motifs containing four invariant aspartate residues. In addition, according to domain analysis, TON-HolPase lacks the imidazoleglycerol-phosphate dehydratase domain of typical DDDD-family members, indicating that it is a monofunctional HolPase. Consequently, TON-HolPase is the first monofunctional HolPase that belongs to the DDDD superfamily. Here, we report the overexpression, crystallization and preliminary X-ray crystallographic analysis of TON-HolPase as a first step toward structure determination.

2. Materials and methods

2.1. Expression and purification of TON-HolPase

To express TON-HolPase in a soluble form, the TON_0887 gene was amplified by the polymerase chain reaction and inserted down-

Table 1
Crystal information and data-collection statistics.

Values in parentheses are for the outer shell (2.28–2.2 Å).

Space group	$P222_1$
Unit-cell parameters (Å)	a = 40.88, b = 46.89, c = 148.03
Wavelength (Å)	0.96405
Resolution (Å)	50–2.2
Completeness (>0σ) (%)	95.1 (84.8†)
$R_{\rm merge}$‡ (%)	9.3 (32.3)
Average $I/\sigma(I)$	8.9 (2.7)
Unique reflections	14419
Average redundancy	4.1 (2.7)
Mosaicity	0.53

† Many weak reflections in the outer shell are deleted owing to the σ cutoff, which causes the drop in completeness in the outer shell. ‡ $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$.

stream of the T7 promoter of the expression plasmid pET-24a (Novagen) as described previously (Lee, Cho *et al.*, 2008). The resulting construct expresses residues 1–240 of the TON-HolPase protein with an additional 15 residues including six histidines at the C-terminus (VDKLAAALEHHHHHH). After verifying the DNA sequence, plasmid DNA was transformed into *E. coli* strain Rosetta (DE3) pLysS (Stratagene). The transformed cells were grown in Luria–Bertani medium (Merck) containing 50 µg ml^{-1} kanamycin to an OD_{600} of 0.5 at 310 K and the expression of TON-HolPase was induced with 1 m*M* isopropyl β-D-1-thiogalactopyranoside (Duchefa). After 12 h induction at 303 K, the cells were harvested and resuspended in 50 m*M* Tris–HCl pH 8.0. The cells were disrupted by sonication and the crude lysate was centrifuged at 20 000*g* for 60 min at 277 K. The resulting supernatant was loaded onto an Econo-Column chromatography column (Bio-Rad) packed with 20 ml nickel-nitrilotriacetic acid (Ni–NTA) resin (Qiagen). The column was washed with a washing buffer containing 50 m*M* Tris–HCl pH 8.0 and 10 m*M* imidazole. TON-HolPase was eluted with the same buffer containing 300 m*M* imidazole. The ∼50 ml eluted fraction containing the TON-HolPase protein was concentrated to 5 ml and subsequently loaded onto a Superdex 75 HR 16/60 column (Amersham Biosciences) pre-equilibrated with a buffer containing 50 m*M* Tris–HCl pH 8.0, 1 m*M* dithiothreitol (DTT) and 150 m*M* NaCl. The TON-HolPase protein was eluted at ∼45 min with a flow rate of 1.5 ml min^{-1}. The purified TON-HolPase was dialysed against a buffer containing 50 m*M* Tris–HCl pH 8.0 and 3 m*M* DTT and then concentrated to ∼26 mg ml^{-1} using a centrifugal concentrator (Vivaspin 20, Sartorius) for crystallization. The protein concentration was measured using the absorbance at 280 nm and the molar absorption coefficient of TON-HolPase (1.403).

2.2. Microbatch crystallization and X-ray data collection

Crystal screening was performed with all the available screening kits from Hampton Research using the microbatch crystallization method at 295 K as described previously (Lee *et al.*, 2004). Small drops composed of 1 µl protein solution and an equal volume of crystallization reagent were pipetted under a layer consisting of a 1:1 mixture of silicon oil and paraffin oil in 72-well HLA plates (Nunc). Initial crystals of TON-HolPase were grown using a precipitant solution containing 12%(*w*/*v*) polyethylene glycol 6000 (PEG 6K), 0.1 *M* magnesium chloride hexahydrate, 0.1 *M* *n*-(2-acetamide)-iminodiacetic acid pH 6.5 (condition No. 25 of MembFac from Hampton Research; Fig. 1). It took over four months to obtain initial thin plate-shaped crystals and we therefore tried to collect a native data set using the initial crystals. Since the thin plate-shaped crystals were conglomerated, for data collection we separated a single crystal using Micro-Tools from Hampton Research. A separated crystal of ∼100 × 50 × 10 µm in size was mounted using a nylon loop (10 micron Mounted CryoLoop from Hampton Research) for data collection after a brief soak in a cryoprotectant solution consisting of 12% PEG 6K, 0.1 *M* magnesium chloride hexahydrate, 0.1 *M* *n*-(2-acetamide)iminodiacetic acid pH 6.5 and 20% glycerol. A 2.2 Å resolution native data set was collected from flash-cooled crystals in a cold N_2 stream at 100 K using an ADSC Quantum 270 CCD on the micro-focused beamline PF-17A of the Photon Factory, Japan (Table 1). The exposure time to the synchrotron radiation was 3 s. A total of 180 frames of 1° oscillation were measured with the crystal-to-detector distance set to 255 mm. Diffraction data were processed using *DENZO* and scaled using *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

Investigation of systematic absences in the reflections showed that the crystals of TON-HolPase belonged to the orthorhombic space group $P222_1$, with unit-cell parameters a = 40.88, b = 46.89, c = 148.03 Å. The calculated unit-cell volume per unit of molecular mass ($V_{\rm M}$) is 2.38 Å^3 Da^{-1} with a solvent content of 48.31% by volume (Matthews, 1968) when one unit cell is assumed to contain four molecules. This corresponds to one molecule per asymmetric unit. The statistics of data collection are summarized in Table 1.

To date, two HolPases have been structurally studied. The crystal structure of a 267-residue HolPase from *Thermus thermophilus* HB8 (Omi *et al.*, 2007) revealed that PHP-type monofunctional HolPases have a distorted $(\beta\alpha)_7$-barrel fold, while the crystal structure of a 167-residue HolPase domain from *E. coli* (Rangarajan *et al.*, 2006) showed that the HolPase domains of the bifunctional enzymes of the DDDD superfamily adopt an α/β-hydrolase fold that is reminiscent of the haloacid dehalogenase enzymes. The structural discrepancy between the two types of HolPases indicates that their detailed reaction mechanisms are distinct from each other even though they mediate the same reaction. TON-HolPase is likely to adopt an α/β-hydrolase fold since it belongs to the DDDD superfamily. However, considering its length and amino-acid composition, the primary structure of the 240-residue TON-HolPase shows only 17% sequence identity to that of the 167-residue HolPase domain from *E. coli*, indicating that the structure of TON-HolPase would be expected to differ significantly from that of the *E. coli* HolPase domain. Consistent with this, although we attempted to solve the crystal structure of

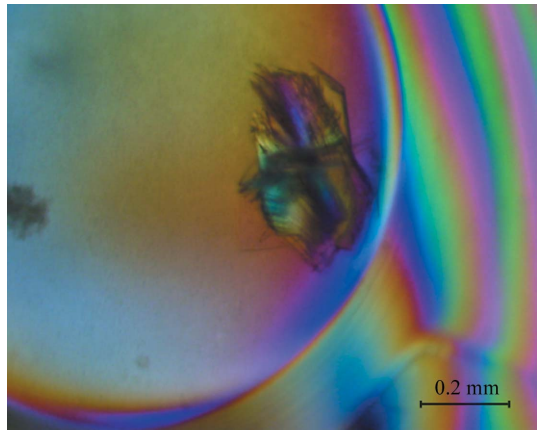


Figure 1
Crystals of TON-HolPase.

TON-HolPase using the molecular-replacement method with the structure of the *E. coli* HolPase domain (PDB code 2fpr) as a search model, all trials using *AMoRe* (Navaza, 2001) and *MOLREP* (Vagin & Teplyakov, 2000) resulted in failure. The crystal structure of TON-HolPase will provide insight into the diversification of HolPases in their structure and catalytic mechanism. Therefore, we are attempting to grow crystals of selenomethionine-substituted TON-HolPase protein in order to solve the crystal structure using the multiple-wavelength anomalous diffraction method.

This study was supported by the Marine and Extreme Genome Research Center Program from the Ministry of Land, Transport and Maritime Affairs and the KORDI in-house program (PE98402).

References

Brilli, M. & Fani, R. (2004). *J. Mol. Evol.* **58**, 225–237.
Chiariotti, L., Nappo, A. G., Carlomagno, M. S. & Bruni, C. B. (1986). *Mol. Gen. Genet.* **202**, 42–47.
Coq, D. le, Fillinger, S. & Aymerich, S. (1999). *J. Bacteriol.* **181**, 3277–3280.
Lee, H. S., Cho, Y., Lee, J.-H. & Kang, S. G. (2008). *J. Bacteriol.* **190**, 2629–2632.
Lee, H. S., Kang, S. G. *et al.* (2008). *J. Bacteriol.* **190**, 7491–7499.
Lee, S.-J., Kim, J. Y., Jung, H. I., Suh, P.-G., Lee, H.-S., Lee, S. H. & Cha, S.-S. (2004). *Acta Cryst.* D**60**, 382–384.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. (2001). *Acta Cryst.* D**57**, 1367–1372.
Omi, R., Goto, M., Miyahara, I., Manzoku, M., Ebihara, A. & Hirotsu, K. (2007). *Biochemistry*, **46**, 12618–12627.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rangarajan, E. S., Proteau, A., Wagner, J., Hung, M. N., Matte, A. & Cygler, M. (2006). *J. Biol. Chem.* **281**, 37930–37941.
Thaller, M. C., Schippa, S. & Rossolini, G. M. (1998). *Protein Sci.* **7**, 1647–1652.
Vagin, A. & Teplyakov, A. (2000). *Acta Cryst.* D**56**, 1622–1624.