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

Purification, crystallization and preliminary X-ray diffraction analysis of the putative ABC transporter ATP-binding protein from *Thermotoga maritima*

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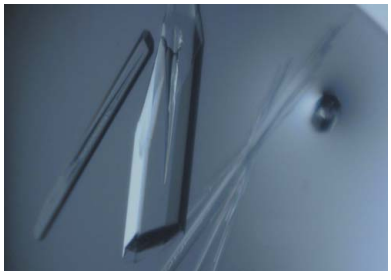
Received 25 December 2007
Accepted 8 May 2008



Adenosine triphosphate (ATP) binding cassette transporters (ABC transporters) are ATP hydrolysis-dependent transmembrane transporters. Here, the overproduction, purification and crystallization of the putative ABC transporter ATP-binding protein TM0222 from *Thermotoga maritima* are reported. The protein was crystallized in the hexagonal space group $P6_422$, with unit-cell parameters $a = b = 148.49$, $c = 106.96$ Å, $\gamma = 120.0^\circ$. Assuming the presence of two molecules in the asymmetric unit, the calculated V_M is 2.84 Å^3 Da^{-1}, which corresponds to a solvent content of 56.6%. A three-wavelength MAD data set was collected to 2.3 Å resolution from SeMet-substituted TM0222 crystals. Data sets were collected on the BL38B1 beamline at SPring-8, Japan.

1. Introduction

The adenosine triphosphate (ATP) binding cassette transporters (ABC transporters) are multidomain integral membrane proteins that translocate a wide range of substrates across cellular membranes (Holland & Blight, 1999; Jones & George, 1999). ABC transporters are one of the four major gene families present in humans (Tatusov *et al.*, 1997) and are also widespread in many organisms (Higgins, 1992). These proteins have enormous implications in medicine. The activity of these proteins is essential for cells in multidrug resistance (Gros *et al.*, 1986; van Veen & Konings, 1998), antigen processing (Schmitt & Tampé, 2000) and virus persistence (Klein *et al.*, 1999; Abele & Tampé, 1999) as well as in pathophysiological conditions such as bare lymphocyte syndrome. The X-ray crystal structures of several full-length transporter proteins from this family have been solved, including those of the putative molybdate transporter ($ModB_2C_2$) from *Archaeoglobus fulgidus* in complex with its binding protein (Hollenstein *et al.*, 2007), Sav1866 from *Staphylococcus aureus* (Dawson & Locher, 2006), the HI1470/1 transporter from *Haemophilus influenzae* (Pinkett *et al.*, 2006) and the BtuCD ABC transporter from *Escherichia coli* (Locher *et al.*, 2002). The ABC transporters share a common architecture composed of four domains: two basic transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). The domains differ in their relative orientations with respect to each other in different transporters. The TMDs contain multiple hydrophobic segments that span the membrane. Structural studies have revealed that the nucleotide-binding domain (NBD) is composed of a catalytic subdomain harbouring the Walker A and Walker B motifs and a helical subdomain that contains the ABC signature motif (Schmitt & Tampé, 2002). Although Walker A and Walker B motifs have been observed in other proteins that hydrolyze ATP, the 'signature motif', also called the 'LSGGQ motif', is specific to the ABC transporters. The TMD has a substrate-binding site that is formed across the membrane. The NBDs are present in the cytoplasm and transform the energy of ATP hydrolysis into conformational changes of the protein. The NBD is connected to the two TMDs through a hydrophilic NBD loop. Of the two different domains present in the ABC transporter protein, the TMDs are markedly more variable compared with the NBDs. Although a number of structures of ABC proteins have been deter-

mined, many fundamental questions about their mechanism of action still remain unanswered. We now report the crystallization and preliminary diffraction analysis of the putative ABC transporter ATP-binding protein TM0222 from *Thermotoga maritima*.

2. Materials and methods

2.1. Cloning, expression, and purification.

The gene encoding TM0222 (gi:56748893) was amplified *via* PCR using *T. maritima* MSB8 genomic DNA and was cloned into the pET-21a expression vector (Novagen, Madison, Wisconsin, USA). The expression vector was introduced into the *E. coli* BL21-CodonPlus(DE3)-RIL-X strain (Stratagene, La Jolla, California, USA) and the recombinant strain was cultured in minimal medium containing 25 µg ml^{-1} selenomethionine, 30 µg ml^{-1} chloramphenicol and 50 µg ml^{-1} ampicillin. The cells (9.9 g) from 3 l of medium were collected by centrifugation, washed with 50 ml Tris–HCl pH 8.0 containing 0.5 *M* NaCl and resuspended in 25 ml of the same buffer. The cells were disrupted by sonication in a chilled water bath and the cell lysate was incubated at 343 K for 10 min. The sample was then centrifuged at 15 000*g* for 30 min and the supernatant was applied onto a Resource ISO column (GE Healthcare Biosciences) equilibrated with 50 m*M* sodium phosphate buffer pH 7.0 containing 1.5 *M* ammonium sulfate and was eluted with a linear (1.5–0 *M*) gradient of ammonium sulfate. The target sample, which eluted in the 0.65 *M* ammonium sulfate fraction, was then applied onto a Resource Q column (GE Healthcare Biosciences) equilibrated with 20 m*M* Tris–HCl pH 8.0 and eluted with a linear gradient of 0–0.5 *M* NaCl. The fractions that eluted in 0.135 *M* NaCl were further purified using a hydroxyapatite CHT10-I column (Bio-Rad) with a linear gradient of 10–500 m*M* sodium phosphate buffer pH 7.0 containing 150 m*M* NaCl. The fractions containing the TM0222 protein were collected and applied onto a HiLoad 16/60 Superdex 75pg column (GE Healthcare Biosciences) equilibrated with 20 m*M* Tris–HCl pH 8.0 containing 150 m*M* NaCl. The protein sample was analyzed by SDS–PAGE (Fig. 1) and was confirmed by N-terminal amino-acid sequencing. After concentration to 15.2 mg ml^{-1} by ultrafiltration, the protein yield was 23.8 mg from 9.9 g of cells.

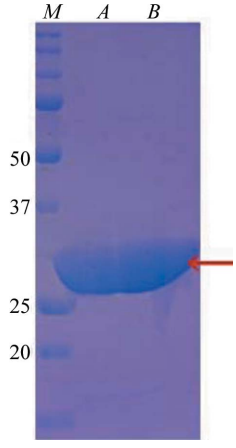


Figure 1
Purification of the putative ABC transporter ATP-binding protein TM0222: SDS–PAGE profile of the purified putative ABC transporter ATP-binding protein. Lane *M*, molecular-weight markers. The size of each protein band is shown on the left in kDa. Lanes *A* and *B* (same sample), purified TM0222 fraction. The electrophoresis was performed with a 17.5% SDS polyacrylamide gel, which was stained with Coomassie Brilliant Blue.

Table 1
Crystallographic data statistics for data reduction and MAD phasing.

Values in parentheses are for the last shell.

	Se peak	Se remote	Se edge
Unit-cell parameters (Å, °)	$a = b = 148.49$, $c = 106.96$, $\gamma = 120.0$		
Space group	$P6_422$		
Beamline	BL38B1, SPring-8		
Wavelength (Å)	0.97891	0.90000	0.97934
Resolution (Å)	50.0–2.30	50.0–2.40	50.0–2.40
Total reflections	2563021	2093737	1613429
No. of unique reflections	31555	27942	27797
Redundancy	21.3 (17.8)	21.2 (17.1)	20.4 (13.2)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	99.8 (97.5)
$R_{\rm merge}$† (%)	8.8 (42.0)	8.0 (48.3)	8.1 (46.8)
Mean $I/\sigma(I)$	25.29 (3.73)	22.81 (3.18)	21.9 (2.06)

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

2.2. Protein crystallization

Crystallization was performed by the sitting-drop vapour-diffusion method at 293 K using Linbro multiwell plates. Each drop, consisting of 1.0 µl of a 15.2 mg ml^{-1} protein solution in 20 m*M* Tris–HCl pH 8.0 containing 150 m*M* NaCl and 1 µl reservoir solution, was allowed to equilibrate against 100 µl reservoir solution. Preliminary screenings were performed using the Hampton Research PEG/Ion Screen kit. Small crystals appeared with a reservoir solution consisting of 0.2 *M* potassium fluoride in 20%(*w*/*v*) PEG 3350 pH 7.3. After optimization, large crystals were obtained at 293 K from a reservoir solution containing 0.2 *M* potassium fluoride and 20%(*w*/*v*) PEG 3350 pH 7.5. Crystals suitable for X-ray data collection appeared (Fig. 2) within 7 d and reached final dimensions of 1.0 × 0.4 × 0.1 mm.

2.3. X-ray intensity data collection and processing

Diffraction data were collected using a Jupiter 210cs CCD detector on the BL38B1 beamline, SPring-8, Japan. The crystals were flash-cooled in a nitrogen-gas stream at 100 K directly using 20%(*v*/*v*) PEG 400 as a cryoprotectant from a drop made up of equal volumes of the protein solution and reservoir solution containing 0.2 *M* potassium fluoride, 20%(*w*/*v*) PEG 3350 pH 7.5. The crystals were maintained at 100 K during data collection. The crystal-to-detector distance was set to 200 mm. A MAD (multiple anomalous diffraction) data set was collected corresponding to the maximum f'' (peak, 0.97891 Å), the minimum f' (edge, 0.97934 Å) and a reference wavelength (remote, 0.9000 Å) chosen on the basis of the absorption spectrum of the Se atom. The diffraction data (Fig. 3) were processed with the *HKL*-

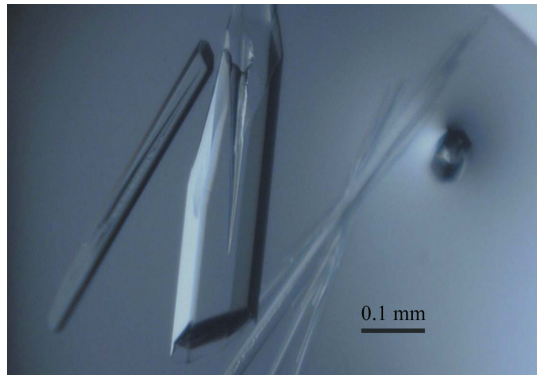


Figure 2
Crystals of the putative ABC transporter ATP-binding protein.

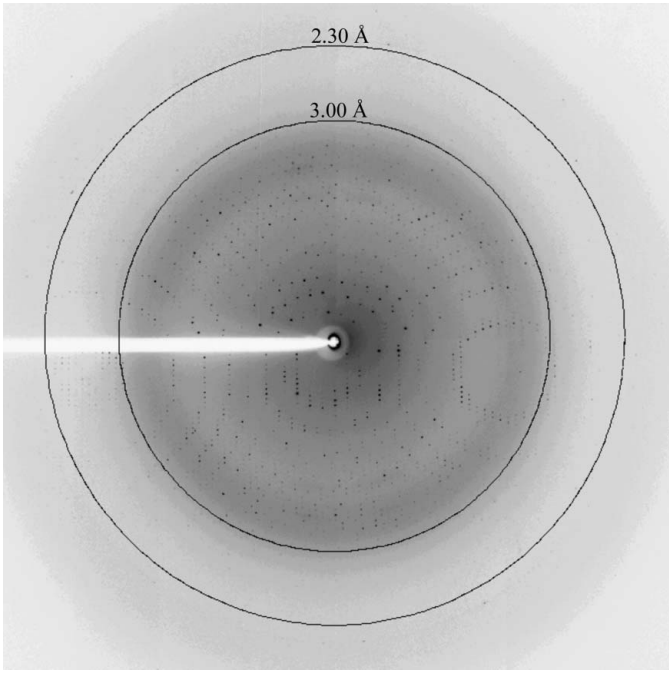


Figure 3
X-ray diffraction pattern of the TM0222 crystal obtained on beamline BL38B1 at SPring-8. The crystal-to-detector distance was 200 mm, the oscillation angle was 1.0° and the exposure time was 10 s.

2000 package (Otwinowski & Minor, 1997). The crystallographic data and the MAD data statistics are summarized in Table 1.

3. Discussion

The TM0222 protein was crystallized by the vapour-diffusion method, yielding hexagonal crystals belonging to space group $P6_422$ with unit-cell parameters $a = b = 148.49$, $c = 106.96$ Å, $\gamma = 120.0°$. Assuming the presence of two molecules (266 amino-acid residues per monomer) in the asymmetric unit, the calculated V_M is 2.84 Å^3 Da^{-1}, which corresponds to a solvent content of 56.6% (Matthews, 1968). These values are within the range usually observed in protein crystals. A high-quality diffraction data set was obtained to 2.30 Å resolution, with 100% completeness and an R_{merge} of 8.8% (Table 1). The highest sequence similarity to other known structures of ABC transporter proteins was 34% for the proteins from *Alicyclobacillus acido-caldarius* (PDB code 1z47; Scheffel *et al.*, 2005) and *Sulfolobus solfataricus* (PDB codes 1oxs, 1oxt, 1oxu, 1oxv; Verdon *et al.*, 2003) and the sugar transporter from *Pyrococcus horikoshii* (PDB code 1v43; Ose *et al.*, 2004). Initial phases were determined by the MAD method using the anomalous signal from the Se atoms. The 12 expected SeMet sites in the structure were obtained with the program *SOLVE* (Terwilliger & Berendzen, 1999) and these peaks were further refined using *RESOLVE* (Terwilliger, 2000, 2003). Using the *PHENIX* automated crystallographic program (Adams *et al.*, 2002), nearly 40% of the residues present in the protein were built. Further model building and refinement are currently under way.

We thank the staff for technical support in the use of beamline BL38B1, SPring-8, Japan. PK, TPS and ASE thank the Council of Scientific and Industrial Research (CSIR), Government of India for funding. We thank Ms M. Nishida and Dr S. Kuramitsu for their help in sample preparation. We are grateful to Ms T. Nakayama and Ms A. Ishii for their clerical assistance. This work was supported in part by the RIKEN Structural Genomics/Proteomics Initiative (RSGI), the National Project on Protein Structural and Functional Analyses, Ministry of Education, Culture, Sports, Science and Technology of Japan.

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