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Purification, crystallization and preliminary X-ray diffraction studies of the ATP-binding subunit of an ABC transporter from *Geobacillus kaustophilus*

ATP-binding cassette (ABC) transporters, also known as traffic ATPases, form a large family of integral membrane proteins responsible for the translocation of a variety of chemically diverse substrates across the lipid bilayers of cellular membranes of both prokaryotes and eukaryotes by the hydrolysis of ATP. The ATP-binding subunit of an ABC transporter from *Geobacillus kaustophilus*, a homodimeric enzyme, was overexpressed in *Escherichia coli* and purified. Crystals were obtained using the microbatch-under-oil method at 291 K. X-ray diffraction data to 1.6 Å resolution were collected on SPring-8 beamline BL26B1. The crystals belonged to the orthorhombic space group *I*222, with unit-cell parameters $a = 54.94$, $b = 78.63$, $c = 112.96$ Å. Assuming the presence of a dimer in the asymmetric unit gave a crystal volume per protein weight (V_M) of $2.32 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 47%; this was consistent with the results of a dynamic light-scattering experiment, which showed a dimeric state of the protein in solution. Molecular-replacement trials using the crystal structure of HisP from the *Salmonella typhimurium* ATP-binding subunit of an ABC transporter as a search model did not provide a satisfactory solution, indicating that the two ATP-binding subunits of ABC transporters have substantially different structures.

1. Introduction

ATP-binding cassette (ABC) transporters represent one of the largest superfamilies of primary transporters. They are found in all phyla of life and are responsible for many physiological processes ranging from solute uptake to multidrug resistance. ABC transporters have a conserved domain structure and consist of two membrane-spanning domains that form the transport pathway and two cytosolic nucleotide-binding domains that energize the transport through the hydrolysis of ATP. The nucleotide-binding domains are highly conserved and contain an amino-acid sequence motif (LSGGQ) that is involved in the binding of nucleotides.

The structures of several full-length ABC transporters (BtuCD from *Escherichia coli*, MsbA from *E. coli* and MsbA from *Vibrio cholerae*) and of many isolated nucleotide-binding domains (NBDs) [HisP from *Salmonella typhimurium* (Hung *et al.*, 1998), ABC transporter from *Methanococcus jannaschii* (Karpowich *et al.*, 2001) and MalK from *E. coli* (Chen *et al.*, 2003)] have been determined. The structures of the NBDs are highly conserved and include an ATP-binding core domain with Walker A and B motifs.

ATP-binding ABC transporters are ubiquitous in biology; they power the translocation of substrates across the membrane, often against a concentration gradient, by hydrolyzing ATP and have been subjected to extensive biochemical and structural studies in recent years. The ATP-binding subunit of an ABC transporter from *Geobacillus kaustophilus*, a 241-residue protein (27 518 Da), shows an amino-acid sequence identity of 26% to the *S. typhimurium* ATP-binding subunit of an ABC transporter. In order to gain insight into the mechanism of the ATP-binding subunit of an ABC transporter, we initiated a structural study. Here, we describe the overexpression, purification and crystallization of the ATP-binding subunit of an

ABC transporter from *G. kaustophilus*. The structure of the ATP-binding subunit will help in understanding the function of the ABC transporter.

2. Experimental

2.1. Protein expression and purification

The ATP-binding subunit of an ABC transporter from *G. kaustophilus* used in this study has a molecular mass of 27 518 Da and consists of 241 amino-acid residues. The plasmid encoding this protein was digested with *Nde*I and *Bgl*II and the fragment was inserted into the expression vector pET-HisTEV (Novagen) linearized with *Nde*I and *Bam*HI. The plasmid contained an N-terminal His tag and a tobacco etch virus (TEV) protease-cleavage site and was expressed in *E. coli* BL21-CodonPlus(DE3)-RIL cells at 310 K. The cells were transformed with the recombinant plasmid and grown without IPTG induction at 310 K in Luria–Bertani medium containing 50 $\mu\text{g ml}^{-1}$ ampicillin for 20 h. The cells were harvested by centrifugation at 4500g for 5 min at 277 K; they were subsequently suspended in 20 mM Tris–HCl pH 8.0 containing 0.5 M NaCl, 5 mM β -mercaptoethanol and were finally disrupted by sonication and heated at 363 K for 10 min. The cell debris and denatured protein were removed by centrifugation (15 000g for 30 min). The supernatant solution was used as the crude extract for purification.

The crude extract was desalted using a HisTrap HP5 desalting column (GE Healthcare), applied onto a Toyopearl SuperQ-650M column (Tosoh) equilibrated with 20 mM Tris–HCl pH 8.0 containing 0.5 M NaCl, 20 mM imidazole (buffer A) and eluted using a gradient of 0.02–0.5 M imidazole. The sample was concentrated by ultrafiltration (Vivaspin) and loaded onto a HiLoad 16/60 Superdex 200 prep-grade column (Amersham Biosciences) equilibrated with buffer A. The His tag was cleaved off by overnight incubation with 1:100 His-tagged TEV protease at 303 K. The protein was again subjected to a HisTrap HP5 desalting column using buffer A. Imidazole was eliminated by dialysis against buffer A. The protein was concentrated by ultrafiltration and loaded onto a HiPrep 26/10 column (GE Healthcare) with 20 mM Tris–HCl pH 8.0 containing 0.2 M NaCl. The protein concentration was measured from the absorbance at 280 nm using a calculated extinction coefficient of 13 900 $\text{M}^{-1} \text{cm}^{-1}$. The homogeneity and the identity of the purified sample were estimated by SDS–PAGE (Laemmli, 1970) and N-terminal sequence analysis.

Figure 1

Crystal of the ATP-binding subunit of an ABC transporter. The crystals have approximate dimensions of $0.1 \times 0.1 \times 0.1$ mm.

The purified ATP-binding subunit of the ABC transporter was finally concentrated by ultrafiltration to 12 mg ml^{-1} in buffer A containing 0.2 M NaCl.

The oligomeric state of the purified ATP-binding subunit of the ABC transporter was examined by a dynamic light-scattering experiment using a DynaPro MS/X instrument (Protein Solutions), which was performed at a protein concentration of 12 mg ml^{-1} in 20 mM Tris–HCl pH 7.6 with 0.2 M NaCl. Several measurements were taken at 291 K and were analyzed using the DYNAMICS software v.3.30 (Protein Solutions). A bimodal analysis resulted in a molecular weight of 51 kDa, which is consistent with the dimeric state of the protein in solution.

2.2. Crystallization

Crystallization screens from Hampton Research (Jancarik & Kim, 1991) were used to determine initial crystallization conditions for the ABC transporter protein. Crystallization was carried out using the microbatch-under-oil method in Nunc HLA plates at 291 K. Drops were made up of equal volumes (1.0 μl) of protein solution and precipitant solution and were overlaid with a 7:3 mixture of silicone and paraffin oils, allowing slow evaporation of the water in the drop. We obtained good crystals using a precipitant solution consisting of 27.5% (w/v) PEG 4000, 0.1 M MES–NaOH, 1 M lithium chloride pH 6.1. The initial crystals were clusters of very small crystals. Several rounds of optimization resulted in single crystals with sharp edges and dimensions of $0.1 \times 0.1 \times 0.1$ mm (Fig. 1). These crystals typically appeared about three weeks after setup.

2.3. Data collection and analysis

The crystals were flash-cooled in a cryoprotectant solution consisting of the precipitant solution diluted with glycerol at 20% (v/v). X-ray diffraction intensity data were collected on SPring-8 beamline BL26B1 using a Jupiter 210 detector (Rigaku). A total of 180 frames were collected with 1° oscillation and 15 s exposure time per image (Fig. 2). The wavelength of the synchrotron radiation was 1.0 Å and the crystal-to-detector distance was 150 mm. The diffraction data

Figure 2

Diffraction pattern of a crystal of the ATP-binding subunit of an ABC transporter.

Table 1

Crystal data and data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	<i>I</i> 222
Unit-cell parameters (Å)	<i>a</i> = 54.94, <i>b</i> = 78.63, <i>c</i> = 112.96
Resolution range (Å)	50.0–1.60 (1.66–1.60)
Total observations	417276
Unique reflections	66397
Multiplicity	6.3 (3.8)
Completeness (%)	99.2 (94.0)
Mean <i>I</i> / σ (<i>I</i>)	10.8 (1.9)
<i>R</i> _{merge} [†] (%)	6.4 (41.4)

[†] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl) \rangle$ are the observed intensity of measurement *i* and the mean intensity of a reflection with indices *hkl*, respectively.

were integrated and scaled to 1.6 Å resolution using *DENZO* and *SCALEPACK* as implemented in the *HKL-2000* program package (Otwinowski & Minor, 1997).

3. Results and discussion

X-ray diffraction data were collected at 110 K to 1.6 Å resolution using synchrotron radiation on beamline BL26B1 at SPring-8, Japan with a CCD detector (Table 1). The crystals belonged to space group *I*222, with unit-cell parameters *a* = 54.94, *b* = 78.63, *c* = 112.96 Å. The calculated Matthews coefficient *V*_M (Matthews, 1968) was 2.3 Å³ Da^{−1}, corresponding to a solvent content of 47%. A dynamic light-scattering experiment showed a result consistent with a dimeric state of this protein in solution (see §2.1). A complete data set was collected and the data-collection statistics are summarized in Table 1. Selenomethionine labelling or soaking the crystals in a solution of NaBr are being considered in order to solve the structure of the ATP-binding subunit of the ABC transporter using multiple isomorphous replacement/anomalous dispersion phasing.

The sequence of the ATP-binding subunit of the ABC transporter was compared with sequences in the Protein Data Bank to obtain a search model for molecular-replacement calculations. The closest homologue in the PDB was HisP, the ATP-binding subunit of histidine permease, an ABC transporter from *S. typhimurium* (PDB entry 1b0u; Hung *et al.*, 1998), which has 26% amino-acid sequence identity. Molecular-replacement trials were performed with *MOLREP* (Vagin & Teplyakov, 2010) using 1b0u as the search model. The top solution had an *R* factor of >0.65 and a correlation coefficient of <0.12, indicating that the two ATP-binding subunits had substantially different structures. Therefore, the structure of the ATP-binding subunit of the ABC transporter will be determined by the multi-wavelength anomalous dispersion method using bromine derivatives as well as selenomethionine-derivative crystals.

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