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Trehalose and maltose uptake in the hyperthermophilic archaeon *Thermococcus litoralis* is mediated by an ABC transport system. The heterotetrameric transport complex $MalFGK_2$, consisting of two membrane-spanning subunits and two copies of an ATP-binding cassette protein, has been crystallized. The crystals belong to the monoclinic space group $C2$, with unit-cell parameters a = 106.5, b = 150.5, c = 170.1 Å, β = 107.8°. A native data set has been obtained at a resolution of 5 Å.

1. Introduction

The trehalose/maltose-transport complex $MalFGK_2$ from *Thermococcus litoralis* belongs to the family of ATP-binding cassette (ABC) proteins. Members of this large superfamily of proteins are ABC transporters that are found in all eubacterial, archaeal and eukaryotic species (Saurin *et al.*, 1999). They share a common architecture, usually consisting of four distinct units, two nucleotide-binding ATPase subunits attached cytoplasmatically to two membrane-spanning domains (Holland & Blight, 1999). Several subclasses can be defined according to the direction of substrate translocation, specificity and subunit organization. Among many others, family members include the mammalian P-glycoprotein (MDR) involved in multidrug resistance, the gated ion-channel cystic fibrosis transmembrane conductance regulator (CFTR), the transporter associated with antigen processing (TAP) and bacterial binding-protein-dependent transport systems.

One of the best studied systems of binding-protein-dependent ABC transporters is the maltose/maltodextrin system of *Escherichia coli* (Boos & Shuman, 1998). In the hyperthermophilic archaeon *T. litoralis*, a highly homologous system for maltose uptake has been discovered (Xavier *et al.*, 1996). The trehalose/maltose-transport complex of *T. litoralis* consists of the two transmembrane-spanning parts MalF (41.6 kDa) and MalG (33.6 kDa), two copies of the nucleotide-binding domain MalK (41.6 kDa) and an extracellular lipid-anchored binding protein TMBP (50.4 kDa).

The number of structures of ABC ATPases is constantly growing and currently known members include HisP (Hung *et al.*, 1998), MalK (Diederichs *et al.*, 2000), MJ1267 (Karpowich *et al.*, 2001), MJ0796 (Yuan *et al.*, 2001) and TAP1 (Gaudet & Wiley, 2001). On the other hand, little is known about the mechanochemical coupling mechanism between the ATPases and the membrane-spanning parts. The first structure of a complete ABC transport complex is the MsbA molecule of *E. coli* determined by Chang & Roth (2001). MsbA is an export complex that forms a homodimer with a V-shaped quaternary arrangement. The structure was solved at 4.5 Å resolution and part of the ATPase domain is disordered.

Recently, Locher *et al.* (2002) published the structure of the vitamin B_{12} transporter $BtuC_2D_2$ from *E. coli* at 3.2 Å resolution. This is the first structure of a complete binding-protein-dependent ABC import complex consisting of four polypeptide chains: two membrane-spanning parts BtuC and two ATP-binding cassettes BtuD, forming a homodimer.

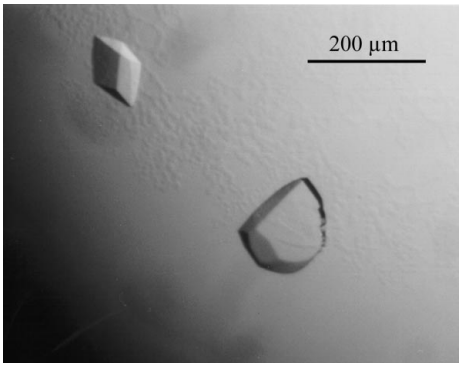

Figure 1
Two single crystals grown in 0.1 *M* sodium cacodylate pH 6.5, 0.4 *M* calcium acetate, 13–14%(*w*/*v*) PEG 4000, 20%(*v*/*v*) glycerol and DDM.

The overall structure shows a relatively compact arrangement with the ATPases in close contact, and are most likely to present one stage of the transport cycle of substrate import.

In this report, we describe the crystallization and preliminary X-ray analysis of $MalFGK_2$, the trehalose/maltose ABC import complex of *T. litoralis*, which consists of a heterodimeric membrane-spanning dimer MalFG and a dimer of the C-terminally elongated ATPase MalK.

2. Materials and methods

2.1. Expression and purification

For overexpression of the $MalFGK_2$ complex in *E. coli* strain TG-1, a two-vector system was used, one coding for the membrane domains and one for the ABC domain, as described by Greller *et al.* (2001). Cells were grown in batch culture with NZA medium, induced with 0.1 m*M* isopropyl thio-β-D-galactopyranoside at OD_{600} = 0.8 and harvested after 4 h. The preparation of the membranes was performed according to the procedure described by Greller *et al.* (2001).

In order to find optimal conditions for crystallization, the $MalFGK_2$ complex was purified in seven different detergents. Solubilization was carried out using 1%(*w*/*v*) *N*,*N*-dimethyldodecylamine-*N*-oxide (LDAO), *N*,*N*-dimethyldecylamine-*N*-oxide (DDAO), *n*-dodecyl-β-D-maltopyranoside (DDM) or 2%(*w*/*v*) *n*-octyl-β-D-glucopyranoside (OG). In the cases of *n*-decyl-β-D-maltopyranoside (DM), *n*-octyl-β-D-maltopyranoside (OM) or cyclohexylhexylmaltoside (CHHM), the membranes were solubilized with 1%(*w*/*v*) DDM. After 1 h solubilization, the solution was heated to 323 K for 20 min. This led to the precipitation of most of the *E. coli* proteins, which were removed by centrifugation at 100 000*g* for 20 min. The yellow supernatant was loaded onto an Ni–NTA column, washed with either 80 ml of buffer *A* (50 m*M* Tris–HCl pH 7.5, 500 m*M* NaCl, 5 m*M* $MgCl_2$, 20 m*M* imidazole) with DDM, LDAO, DDAO or OG at the final concentrations given in Table 1, or washed with 160 ml of buffer *A* containing the detergents DM, OM or CHHM at the final concentrations given in Table 1. In the latter case, the flow rate was set to $\leq$0.2 ml min^{-1} in order to replace DDM with another detergent. The concentrations of all detergents used for solubilization and purification are summarized in Table 1. Afterwards, the protein was eluted with 100 m*M* imidazole in buffer *A* with the detergent used for washing. In a further purification step, the transporter was loaded onto a red Sepharose column with affinity to the ATPase domains and was eluted with a linear gradient (0.5–2.0 *M*) of NaCl. Fractions containing $MalFGK_2$ were pooled (98% pure as judged by SDS–PAGE), concentrated and dialyzed in buffer *B* (50 m*M* Tris pH 7.5, 500 m*M* NaCl, 5 m*M* $MgCl_2$) to lower the NaCl concentration for crystallization. ATPase activity was not tested during purification because transport activity of the $MalFGK_2$ complex in detergent solution cannot be measured, as discussed by Greller *et al.* (2001). The protein concentration was determined by absorption at 280 nm using a theoretically calculated extinction coefficient ε_{calc} = 129 700 $M^{-1}cm^{-1}$. Finally, the crystallization solution contained 2.5–3 mg ml^{-1} $MalFGK_2$ in buffer *B* with one of the detergents at the final concentration listed in Table 1.

Table 1
Detergents used for solubilization and purification of the $MalFGK_2$ complex, with the final concentration in the protein solution used for crystallization.

For purification in DM, OM and CHHM, the membranes were solubilized using DDM, marked by *.

Detergent	Solubilization [%(*w*/*v*)]	Final concentration [%(*w*/*v*)]
n-Dodecyl-β-D-maltopyranoside (DDM)	1.0	0.05
n-Decyl-β-D-maltopyranoside (DM)	1.0*	0.2
n-Octyl-β-D-maltopyranoside (OM)	1.0*	1.2
Cyclohexylhexylmaltoside (CHHM)	1.0*	0.1
n-Octyl-β-D-glucopyranoside (OG)	2.0	1.0
N,*N*-dimethyldodecylamine-*N*-oxide (LDAO)	1.0	0.1
N,*N*-dimethyldecylamine-*N*-oxide (DDAO)	1.0	0.5

2.2. Crystallization and data collection

Initial screening was performed using Screen I (Jancarik & Kim, 1991), Screen II and Membfac (Hampton Research) at 291 K. Firstly, small crystals were obtained in Screen I conditions 42 and 46. Further fine screening yielded regular but very thin platelets, which grew thicker in the third dimension after further improvement.

No significant differences were observed between crystallization conditions, crystal shape and properties upon purification with different detergents.

For cryocooling of the crystals the following cryoprotectants were tested: glycerol, ethylene glycol, PEG 200, PEG 400, sucrose, trehalose in buffer *B* (containing detergent) or dried paraffin oil. All these solutions damaged the crystals if they were directly transferred into them. Better results were achieved when the cryoprotectant was added to the crystallization solution. The final presaturation conditions for crystallization were 0.1 *M* sodium cacodylate pH 6.5, 0.4 *M* calcium acetate, 13–14%(*w*/*v*) PEG 4000, 20%(*v*/*v*) glycerol mixed with the $MalFGK_2$ complex in buffer *B* containing DDM. The only visible differences in the case of the crystals grown in 20%(*v*/*v*) glycerol were the slightly round edges. The crystals shown in Fig. 1 had maximum dimensions of 250 × 150 × 100 µm and grew within 5 d. 2 h before flash-cooling of the crystals 100 µl of glycerol (100%) was added to the reservoir (1 ml). The crystals were then directly transferred into liquid nitrogen.

Data collection was carried out at the EMBL Outstation, DESY at beamlines X13 and BW7B equipped with MARCCD and MAR345 imaging-plate detectors, respectively. Crystals diffracted to a resolution of

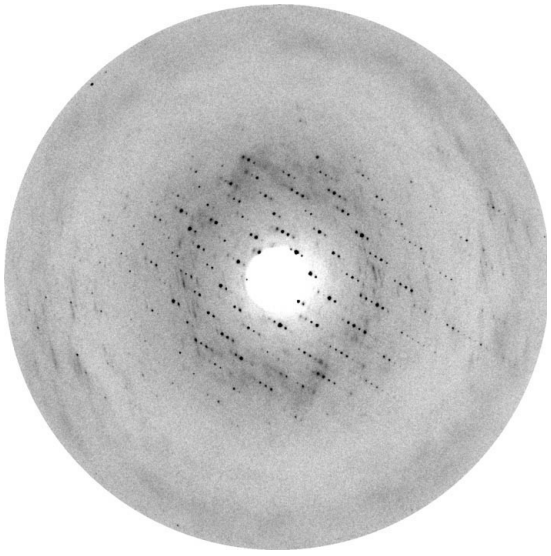

Figure 2
Diffraction image collected at BW7B at the EMBL Outstation, DESY, Hamburg. The resolution at the edge is at 3.8 Å; some single reflections could be observed to 4 Å.

Table 2
Data-collection statistics for a $MalFGK_2$ crystal.

Values in parentheses refer to the resolution shell 6–5 Å.

Space group	*C*2
Unit-cell parameters (Å, °)	a = 106.5, b = 150.5, c = 170.1, β = 107.8
Wavelength (Å)	0.8456
Resolution (Å)	5
Reflections observed	67990
Unique reflections	11093
Completeness (%)	97.3 (96.8)
$I/\sigma(I)$	15.5 (3.54)
R_{sym} (%)	14.7 (53.8)

5 Å; some single reflections could be observed to 4 Å. Fig. 2 shows a diffraction pattern collected at BW7B. The data were processed using *XDS* (Kabsch, 1993).

3. Results and discussion

Initial conditions for crystallization were found by screening experiments. Further improvement indicated that the pH range 6.0–7.0 is suitable for crystallization with no significant difference in crystal quality. Crystal growth was more sensitive to the PEG chain length. PEG 4000 was found to be optimal and mixtures of PEG 8000 and PEG 1000 also worked well. Addition of nucleotides and their analogues, *e.g.* ATP, ADP, ADPNP and ADP-Vi, and various detergents seemed to have no strong influence on crystal quality. Crystallization in the presence of 20%(*v*/*v*) glycerol in the reservoir solution allowed direct cryocooling of the crystals without chemical stress arising from transfer into another solution. Dissolved crystals showed the same pattern on SDS gels as the protein solution used in the crystallization setups.

A data set of 97.3% completeness could be collected to 5 Å resolution, with some reflections extending to 4 Å, at a wavelength λ = 0.8456 Å and an oscillation range of 1°. The results of the data collection are summarized in Table 2. Data analysis showed that $MalFGK_2$ crystallizes in the monoclinic space group *C*2, with unit-cell parameters a = 106.5, b = 150.5, c = 170.1 Å, β = 107.8°. Assuming a molecular weight of 147.6 kDa and one molecule per asymmetric unit, the V_M value is 4.4 Å^3 Da^{-1} with a solvent content of 72%, which is in the normal range for a membrane protein surrounded by a detergent micelle. The diffraction image in Fig. 2 shows that the crystals diffract anisotropically; the lattice order appears to be much better along the *c* axis than along the *a* and *b* axes.

We are now working on the improvement of the crystal quality and the phase determination by experimental methods.

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