crystallization communications

Crystallization and preliminary X-ray diffraction analysis of the full-length Mg^{2+} transporter MgtE

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The MgtE family of Mg^{2+} transporters are ubiquitously conserved in all three domains. The genes encoding full-length MgtE from seven different species were cloned. Three of the seven MgtE transporters were overexpressed and purified for use in crystallization trials. Only *Thermus thermophilus* MgtE was successfully crystallized using the sitting-drop vapour-diffusion method. Selenomethionine-substituted (SeMet) crystals were obtained by cross-micro-seeding using the native microcrystals. The SeMet crystals diffracted X-rays to 3.5 Å resolution using synchrotron radiation and belong to space group $C222_1$, with unit-cell parameters $a = 118.3$, $b = 134.9$, $c = 366.2$ Å. Structure determination is in progress.

1. Introduction

The magnesium ion, Mg^{2+}, is one of the most abundant divalent cations in biological systems and is vital to all living organisms. Three distinct classes of Mg^{2+} transporters have been cloned from bacteria and archaea: CorA, MgtA/B and MgtE (Maguire, 2006). Crystal structures of the CorA Mg^{2+} transporter have been reported (Lunin *et al.*, 2006; Eshaghi *et al.*, 2006; Payandeh & Pai, 2006). The MgtA/B Mg^{2+} transporters belong to the P-type ATPase superfamily, from which the crystal structure of the sarcoendoplasmic Ca^{2+}-ATPase has been solved (Toyoshima *et al.*, 2000). The coordinates of the cytosolic domain of *Enterococcus faecalis* MgtE including Mg^{2+} have recently been deposited in the Protein Data Bank by a structural genomics group (PDB code 2oux; R. Sugadev, M. Sauder, S. K. Burley & S. Swaminathan, unpublished work). However, the full-length structure of an MgtE Mg^{2+} transporter has not yet been determined.

The MgtE-family Mg^{2+} transporters are ubiquitously distributed in all three domains of life (Townsend *et al.*, 1995; Smith *et al.*, 1995; Wabakken *et al.*, 2003). In eubacteria and archaea, MgtE is as widespread as CorA; some species possess either MgtE or CorA, while many species possess both transporters. In addition, MgtE is more widespread than MgtA/B. MgtE consists of a large N-terminal cytosolic domain followed by five C-terminal predicted transmembrane (TM) domains. The cytosolic domain includes a cystathionine-β-synthase (CBS) domain, which is known to play a regulatory function in other transporter proteins, *e.g.* human chloride channels and the osmoregulated ABC transporter OpuA (Ignoul & Eggermont, 2005; Bennetts *et al.*, 2005; Biemans-Oldehinkel *et al.*, 2006). Recently, two mammalian homologues of MgtE, SLC41A1 and SLC41A2, were functionally characterized and suggested to be involved in magnesium homeostasis (Goytain & Quamme, 2005*a*,*b*; Sahni *et al.*, 2007). The SLC41 proteins, which lack the cytosolic domain, have two tandemly repeated domains (N-terminal D1 and C-terminal D2 domains) that are highly homologous to the integral membrane part (from the centre of the predicted TM1 helix to TM5) of the bacterial MgtE proteins. Therefore, bacterial MgtE is expected to be a functional dimer. However, the MgtE transporters have not been thoroughly characterized. Transcriptional regulation of MgtE has not been examined and the mechanism of Mg^{2+} transport and its regulation by MgtE are also unknown.

In order to clarify the mechanism of Mg^{2+} transport by MgtE, the atomic structure of the full-length MgtE is required. Here, we report
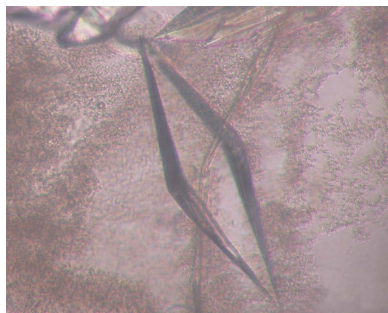
the crystallization of the full-length MgtE from *Thermus thermophilus*, crystals of which diffracted X-rays to 3.5 Å resolution.

2. Materials and methods

2.1. Cloning, expression and purification

The genes encoding MgtE from *Thermus thermophilus* (TTHA1060), *Thermotoga maritima* (TM1161), *Thermobifida fusca* (Tfu_2199), *Bacillus subtilis* (Bsu1332), *Pyrococcus horikoshii* (PH0703), *Methanosarcina mazei* (Mmar10_1742) and *Methanothermobacter thermautotrophicus* (MTH620) were PCR-amplified from genomic DNA and cloned into a pET28a (Novagen) derivative including an N-terminal hexahistidine tag and a Prescission Protease (GE Healthcare) site. Native proteins were expressed in *Escherichia coli* C41 (DE3) (Avidis) cells harbouring pRARE (Novagen) grown in LB medium containing kanamycin (50 µg ml^{-1}) and were induced at an absorbance at 600 nm (A_{600}) of ∼0.5 with 0.5 mM isopropyl β-thiogalactopyranoside for 20 h at 293 K. Selenomethionine-substituted (SeMet) protein was similarly expressed in the methionine auxotroph C41 (DE3) grown in Core medium (Wako) with 30 µg ml^{-1} L-selenomethionine (Nakalai Tesque).

All the MgtE proteins were purified using the following procedure at 277 K; for the Cys-containing proteins all purification buffers included 3 mM β-mercaptoethanol. After sonication in 50 mM HEPES buffer pH 7.0 containing 150 mM NaCl and a protease-inhibitor cocktail (Nakalai Tesque), the disrupted cells were harvested by centrifugation and ultracentrifugation. The membrane fraction was then solubilized for 2 h at 277 K with 50 mM HEPES pH 7.0 buffer containing 2%(w/v) *n*-dodecyl-β-maltoside (DDM; Nakalai Tesque), 150 mM NaCl and 20 mM imidazole. After the removal of insoluble materials by ultracentrifugation, the supernatants were applied onto a Ni–NTA (Qiagen) column equilibrated with buffer *A* [50 mM HEPES pH 7.0, 150 mM NaCl, 0.1%(w/v) DDM] containing 20 mM imidazole. The column-bound proteins were washed and eluted in buffer *A* containing 50 and 300 mM imidazole, respectively.

For further purification, the MgtE fractions were concentrated using an Amicon Ultra 30K filter (Millipore) and applied onto a HiLoad 16/60 Superdex 200 (GE Healthcare) size-exclusion column equilibrated with 20 mM HEPES pH 7.0 buffer containing 150 mM NaCl and one of the following detergents: 0.1% DDM, 0.25% *n*-decyl-β-D-maltoside (Nakalai Tesque), 0.2% C$_{12}$E$_8$ (Anatrace) or 1.25% *n*-octyl-β-D-glucopyranoside (Nakalai Tesque). The purified MgtE proteins were concentrated to approximately 10 mg ml^{-1} using an Amicon Ultra 30K filter for crystallization trials.
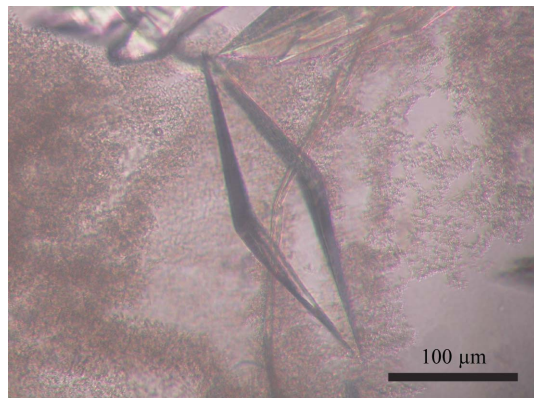
2.2. Crystallization

Initial screening for crystallization conditions was performed using several screening kits purchased from Hampton Research, JB Screen kits (Jena Bioscience) and MemSys and MemStart kits (Molecular Dimensions). A Hydra II Plus One crystallization robot (Matrix Technologies) was used for initial crystallization screening using the sitting-drop vapour-diffusion method at 293 K. In the initial crystallization screen, 0.2 µl protein solution was mixed with 0.2 µl mother liquor. Native crystals of *T. thermophilus* MgtE (0.1 DDM) were first obtained in two weeks from condition No. 16 of the Natrix Screen (Hampton Research). In the optimized crystallization conditions, drops consisting of 1 µl protein solution mixed with 1 µl mother liquor were used in the sitting-drop vapour-diffusion method. After further optimization of the reservoir conditions, tetragonal bipyramidal crystals appeared in two weeks using a reservoir solution containing 26–32% 2-methyl-2,4-pentanediol (MPD), 40 mM magnesium acetate and 100 mM MES pH 6.0. SeMet crystals of *T. thermophilus* MgtE were only obtained under conditions similar to those used for the native protein (32–34% MPD, 40 mM magnesium acetate and 100 mM MES pH 6.0) by the seeding method using seeds prepared from native crystals; the native crystals were crushed with a needle, which was then plunged into fresh crystallization drops containing SeMet protein.

Where indicated, phospholipids (PL) were added to the purified protein prior to crystallization trials by the following procedure: a one-tenth volume of 10 mg ml^{-1} *E. coli* PL polar extract (Avanti Polar Lipids) was added to the purified MgtE protein and the insoluble materials were removed by centrifugation.

2.3. X-ray data collection and processing

All data collections were performed at 100 K in a cold nitrogen stream with an oscillation range per image of 1° using an ADSC Quantum 315 detector on beamline BL41XU at SPring-8. The total oscillation ranges covered were 720° for the peak data set and 180° for other data sets. Before cryocooling, the crystals were cryo-
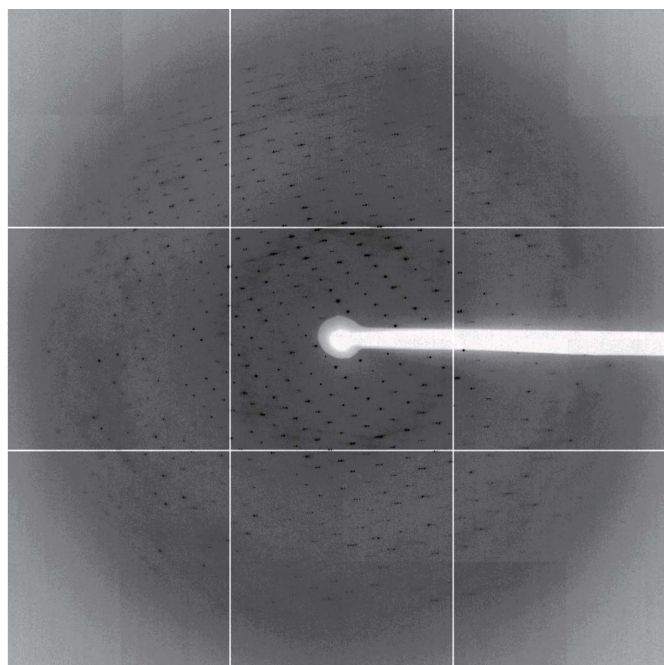

Figure 1
SeMet crystals of *T. thermophilus* MgtE. The scale bar represents 100 µm.

Figure 2
Diffraction patterns of a SeMet crystal of *T. thermophilus* MgtE.

Table 1
Data-collection statistics of SeMet MgtE.

Values in parentheses are for the last shell.

	Peak	Inflection	Low remote
Wavelength (Å)	0.97924	0.97933	0.98200
Space group	$C222_1$		
Unit-cell parameters (Å)	$a = 118.3, b = 134.9, c = 366.2$		
Resolution (Å)	50–3.5 (3.56–3.50)	50–4.0 (4.07–4.00)	50–4.0 (4.07–4.00)
Measured reflections	2349560	311296	328689
Unique reflections	37118	25063	24956
Completeness (%)	99.0 (96.2)	99.1 (94.9)	99.0 (96.7)
$I/\sigma(I)$	8.6 (2.3)	7.2 (3.0)	7.6 (2.6)
R_{merge}†	0.110 (0.453)	0.098 (0.330)	0.095 (0.325)

† $R_{merge} = \sum I_i - \langle I_i \rangle | / \sum \langle I_i \rangle$, where I_i is the observed intensity and $\langle I_i \rangle$ is the average intensity over symmetry-equivalent measurements.

protected with reservoir solutions containing 32–34% MPD, 40 mM magnesium acetate and 100 mM MES pH 6.0. All diffraction data were processed with *DENZO/SCALEPACK* (Otwinowski & Minor, 1997). The data were examined for possible twinning by inspecting the acentric moments of the intensity distributions using the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

To obtain MgtE proteins suitable for crystallization and structure determination, we attempted to express and purify MgtE proteins from seven different species. Three of the seven species, *Thermus thermophilus*, *Thermotoga maritima* and *Thermobifida fusca*, yielded sufficient quantities of MgtE protein for crystallization. The yields of the purified MgtE proteins from *Thermus thermophilus*, *Thermotoga maritima* and *Thermobifida fusca* were about 10, 2 and 2 mg per 10 l of culture, respectively. In the initial crystallization screen, only *T. thermophilus* MgtE (TtMgtE) crystallized in the presence of Mg^{2+}. We note that initially it was difficult to reproduce the crystals; they appeared at a late stage of crystallization with low frequency. Further purification of TtMgtE using anion-exchange chromatography yielded fewer crystals. In contrast, addition of PL (1 mg ml^{-1}) to the purified protein greatly increased the reproducibility of the crystals, with crystals appearing in most of the crystallization drops. These results suggested that the 'overpurified' protein lost PL, which is essential for crystallization. The influence of PL upon membrane-protein crystallization has been reported previously (Zhang *et al.*, 2003; Jidenko *et al.*, 2005; Lemieux *et al.*, 2003; Guan *et al.*, 2006). The cumulative intensity distribution of the native MgtE data appeared to be sigmoidal, which is indicative of twinning, whereas that of the SeMet MgtE data did not (data not shown). Therefore, for phase determination using the multiwavelength anomalous diffraction (MAD) method, a three-wavelength MAD data set was collected from a SeMet MgtE crystal. The SeMet crystals (Fig. 1) diffracted X-rays to 3.5 Å resolution and belong to space group $C222_1$, with

unit-cell parameters $a = 118.3$, $b = 134.9$, $c = 366.2$ Å. A diffraction image is shown in Fig. 2. The mammalian homologues of MgtE have two tandemly repeated TM domains (Wabakken *et al.*, 2003), which allowed us to assume the presence of a dimer in the asymmetric unit. Therefore, the asymmetric unit was expected to contain four MgtE molecules (200.3 kDa), giving a Matthews coefficient V_M of 3.65 Å^3 Da^{-1} and a solvent content of 66.3%. The data-collection statistics are summarized in Table 1. Structure determination using the MAD method is in progress.

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