Yoshiki Tanaka,^a Motoyuki
Hattori,^a Shuya Fukai,^b Ryuichiro
Ishitania^a and Osamu Nureki^{a,c,*}^aDepartment of Biological Information,
Graduate School of Bioscience and
Biotechnology, Tokyo Institute of Technology,
4259 Nagatsuta-cho, Midori-ku, Yokohama-shi,
Kanagawa 226-8501, Japan, ^bCenter for
Biological Resources and Informatics, Tokyo
Institute of Technology, 4259 Nagatsuta-cho,
Midori-ku, Yokohama-shi, Kanagawa 226-8501,
Japan, and ^cSORST, JST, Honcho,
Kawaguchi-shi, Saitama 332-0012, Japan

Correspondence e-mail: nureki@bio.titech.ac.jp

Received 16 May 2007

Accepted 3 July 2007

Crystallization and preliminary X-ray diffraction
analysis of the cytosolic domain of the Mg²⁺
transporter MgtE

The MgtE family of Mg²⁺ transporters is ubiquitously conserved in all domains of life. The cytosolic domains of the MgtE Mg²⁺ transporters include a cystathionine- β -synthase (CBS) domain which is known to play a regulatory function in transporter proteins. The cytosolic domain of MgtE from *Thermus thermophilus* was overexpressed, purified and crystallized in the presence and absence of Mg²⁺. The crystals formed in the presence of Mg²⁺ diffracted X-rays to 2.3 Å resolution using synchrotron radiation, belong to space group *P*6₅22 with unit-cell parameters $a = b = 57.7$, $c = 317.6$ Å and are expected to contain one molecule in the asymmetric unit. The crystals formed in the absence of Mg²⁺ diffracted X-rays to 3.5 Å resolution using synchrotron radiation, belong to space group *P*2₁2₁2₁ with unit-cell parameters $a = 77.0$, $b = 100.3$, $c = 100.3$ Å and are expected to contain two molecules in the asymmetric unit.

1. Introduction

The magnesium ion, Mg²⁺, is one of the most abundant divalent cations in biological systems and is vital to all living organisms. Three distinct classes of Mg²⁺ transporters have been identified in eubacteria and archaea (Nelson & Kennedy, 1971; Maguire, 1992, 2006b; Hmiel *et al.*, 1986; Bui *et al.*, 1999; Kehres & Maguire, 2002; Lunin *et al.*, 2006). The CorA Mg²⁺ transporter acts as the constitutive Mg²⁺-influx system and crystal structures have recently been reported by three groups (Eshaghi *et al.*, 2006; Lunin *et al.*, 2006; Maguire, 2006a). The MgtA and MgtB transporters are in the P-type ATPase superfamily, based on sequence similarity, and act as influx-only systems. The crystal structure of the related P-type sarcomere Ca²⁺ pump has been reported (Toyoshima & Nomura, 2002). In contrast, the MgtE Mg²⁺ transporter, which is ubiquitously conserved in all kingdoms of life (Smith *et al.*, 1995; Townsend *et al.*, 1995; Wabakken *et al.*, 2003; Smith & Maguire, 1998), has not been thoroughly characterized and it is not even clear whether MgtE acts as a channel or an active transporter. Although MgtE is presumed to be an Mg²⁺-influx system, the only actual transport studies on MgtE used Co²⁺ ions, not Mg²⁺ ions (Smith *et al.*, 1995). However, unlike CorA, Ni²⁺ is not transported by MgtE (Smith *et al.*, 1995). The crystal structure of MgtE has not yet been reported. MgtE consists of N-terminal cytosolic domains and a C-terminal transmembrane (TM) domain. The cytosolic domains include a cystathionine- β -synthase (CBS) domain, which reportedly plays a regulatory function in other transporter proteins, *e.g.* human chloride channels and the osmoregulated ABC transporter OpuA (Biemans-Oldehinkel *et al.*, 2006; Bennetts *et al.*, 2005; Ignoul & Eggermont, 2005). Therefore, the cytosolic domains of MgtE are likely to have a regulatory function. During preparation of our manuscript, the coordinates of the cytosolic domain of *Enterococcus faecalis* MgtE including Mg²⁺ (2.16 Å resolution) were 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). Here, we report the crystallization of the cytosolic domain of MgtE from *Thermus thermophilus* in the presence and absence of Mg²⁺.

2. Cloning, expression and purification

The cytosolic domain of MgtE (TtMgtE_{1–275}; $M_r = 31\,245$) from *T. thermophilus* was cloned from genomic DNA into the *Nde*I and

*Bam*HI sites of the pET-15b vector (Novagen). The recombinant protein was overexpressed in C41(DE3) (Avidis) *Escherichia coli* cells grown in LB medium containing ampicillin (100 mg ml^{-1}) at 310 K by induction with 1 mM isopropyl β -D-thiogalactopyranoside (IPTG) for $\sim 16 \text{ h}$ at 293 K when the OD_{600} reached ~ 0.5 . Cells were harvested by centrifugation (6000g, 10 min) and resuspended in sonication buffer (50 mM HEPES pH 7.0, 300 mM NaCl) supplemented with 1 mM phenylmethanesulfonyl fluoride. After sonication of the cells, the crude extract was agitated in a heat bath for 15 min at 343 K. After centrifugation (28 000g) for 15 min, the supernatant was loaded by gravity onto a 10 ml Ni-NTA agarose column (Qiagen) pre-equilibrated in buffer A (50 mM HEPES pH 7.0, 300 mM NaCl) containing 20 mM imidazole, which was extensively washed with buffer A containing 30 mM imidazole. The protein was eluted in buffer A containing 200 mM imidazole and the N-terminal His₆ tag was cleaved using thrombin (Haematologic Technologies) for $\sim 12 \text{ h}$

during dialysis against 50 mM HEPES buffer pH 7.0 containing 20 mM NaCl. The N-terminus of the protein after thrombin cleavage had the expected NH₂-Gly-Ser-His-Met- sequence (the first three residues are derived from the vector). This sample was again applied onto the Ni-NTA column. The flowthrough was fractionated and loaded onto a Mono Q 10/100 GL column (8 ml; GE Healthcare) and the protein was eluted using a gradient of 20–1000 mM NaCl in 20 mM HEPES buffer pH 7.0. The protein eluted from the Mono Q column in a single peak at 450 mM NaCl. Further purification was carried out on a HiLoad 16/60 Superdex 200 (GE Healthcare) gel-filtration column in 20 mM HEPES buffer pH 7.0 containing 150 mM NaCl and the protein eluted in a single peak at an elution volume of 69.1 ml, which corresponds to a molecular weight of $\sim 150 \text{ kDa}$, suggesting oligomerization of TtMgtE_{1–275}. The purified protein was concentrated to about 15 mg ml^{-1} using a centrifugal filter device (Millipore, 5 kDa molecular-weight cutoff) for crystallization

Figure 1
(a) Crystals of TtMgtE_{1–275} in the presence of Mg^{2+} . (b) Crystals of TtMgtE_{1–275} in the absence of Mg^{2+} . The scale bars in (a) and (b) are 300 and 200 μm in length, respectively.

Figure 2
(a) Diffraction pattern of TtMgtE_{1–275} in the presence of Mg^{2+} . (b) Diffraction pattern of TtMgtE_{1–275} in the absence of Mg^{2+} .

Table 1

Data-collection statistics.

Values in parentheses are for the last shell.

	Mg ²⁺ present	Mg ²⁺ present, SeMet	Mg ²⁺ absent	Mg ²⁺ absent, SeMet
Wavelength (Å)	1.0000	0.97911	1.0000	0.97909
Temperature (K)	100	100	100	100
Space group	<i>P</i> 6 ₅ 22	<i>P</i> 6 ₅ 22	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 57.7, <i>c</i> = 317.6, α = β = 90, γ = 120	<i>a</i> = <i>b</i> = 57.8, <i>c</i> = 317.7, α = β = 90, γ = 120	<i>a</i> = 77.0, <i>b</i> = 100.3, <i>c</i> = 100.3, α = β = γ = 90	<i>a</i> = 77.1, <i>b</i> = 100.2, <i>c</i> = 100.2, α = β = γ = 90
Resolution (Å)	50–2.3 (2.34–2.30)	50–2.3 (2.38–2.30)	50–3.5 (3.56–3.50)	50–3.9 (3.97–3.90)
Measured reflections	268641	247439	106648	65842
Unique reflections	14966	14932	10184	7460
Completeness (%)	98.8 (99.3)	98.9 (98.8)	99.0 (96.9)	99.3 (98.4)
Redundancy†	18.0 (16.0)	16.6 (14.0)	10.5 (5.2)	8.8 (5.4)
<i>I</i> /σ(<i>I</i>)	68.6 (8.2)	60.0 (7.4)	49.9 (1.9)	41.9 (2.2)
<i>R</i> _{merge} ‡	0.057 (0.260)	0.083 (0.374)	0.052 (0.406)	0.070 (0.381)

† Bijvoet pairs were not merged in the calculation of redundancy. ‡ *R*_{merge} = Σ|*I*_{*i*} − ⟨*I*⟩|/Σ⟨*I*⟩, where *I*_{*i*} is the observed intensity and ⟨*I*⟩ is the average intensity over symmetry-equivalent measurements.

screening. These procedures reproducibly yielded ~5 mg protein from 1 l bacterial culture. Selenomethionine (SeMet) substituted TtMgtE_{1–275} was overexpressed in B834(DE3) and was purified in the same way as the native protein.

3. Crystallization

Initial screening for crystallization conditions was performed using Crystal Screens 1 and 2, PEG/Ion Screen, Index, Natrix, SaltRX and MembFac kits from Hampton Research and MemSys and MemStart kits from Molecular Dimensions. A Hydra II Plus One crystallization robot (Matrix Technologies) was used for screening using the sitting-drop vapour-diffusion method at 293 K, mixing equal volumes (0.2 µl) of the protein solution (15 mg ml^{−1} in the gel-filtration buffer) and reservoir solution.

Crystals of TtMgtE_{1–275} in the presence and absence of Mg²⁺ were initially obtained from condition No. 23 from Crystal Screen 1 (0.2 M magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 30% PEG 400) and condition No. 9 from PEG/Ion Screen (0.2 M ammonium chloride, 20% PEG 3350), respectively. In the optimized crystallization conditions, 1 µl protein solution (15 mg ml^{−1} in gel-filtration buffer) was mixed with 1 µl mother liquor for vapour diffusion against 0.5 ml reservoir solution.

After further optimization of the crystallization conditions, the best crystals of TtMgtE_{1–275} in the presence of Mg²⁺ were obtained using 18–22% PEG 400, 0.2 M MgCl₂, 0.1 M HEPES pH 7.4. The crystals appeared overnight and grew to maximum dimensions of about 0.5 × 0.2 × 0.2 mm within 2–3 d (Fig. 1*a*). Small crystals of TtMgtE_{1–275} in the absence of Mg²⁺ were obtained in 20% PEG 3350 and 0.2 M ammonium acetate; the use of ammonium acetate instead of ammonium chloride greatly improved the reproducibility of the crystals. However, the apparent mosaicity was high. To obtain larger crystals with lower mosaicity, extensive optimizations of the crystallization conditions were carried out using Additive Screen (Hampton Research). The addition of 0.5% (w/v) octyl β-D-glucopyranoside (β-OG) significantly improved the crystal morphology, as judged by X-ray diffraction. The presence of β-OG yielded a large number of rod-shaped crystals in 2–3 d, which grew to maximum dimensions of about 1.0 × 0.1 × 0.1 mm within a week (Fig. 1*b*). Crystals of SeMet-substituted TtMgtE_{1–275} in the presence and absence of Mg²⁺ were obtained using the same crystallization conditions as were used for the native crystals.

4. Preliminary crystallographic analysis

All TtMgtE_{1–275} X-ray diffraction data sets were collected at 100 K in a cold nitrogen stream using an ADSC Quantum 315 detector on beamline BL41XU at SPring-8 and were processed using *DENZO/SCALEPACK* (Otwinowski & Minor, 1997). Prior to cryocooling in a nitrogen stream, the crystals of TtMgtE_{1–275} in the presence of Mg²⁺ were transferred into a cryoprotectant solution containing 33% PEG 400, 0.2 M MgCl₂ and 0.1 M HEPES pH 7.4, while the crystals of TtMgtE_{1–275} in the absence of Mg²⁺ were transferred into a cryoprotectant solution containing 20% PEG 3350, 0.2 M ammonium acetate, 0.5% β-OG and 10% ethylene glycol.

The crystals of TtMgtE_{1–275} in the presence of Mg²⁺ diffracted to 2.3 Å resolution (Fig. 2*a*) and belonged to space group *P*6₅22 (unit-cell parameters *a* = *b* = 57.7, *c* = 317.6 Å), with the asymmetric unit being likely to contain one molecule. This corresponds to a Matthews coefficient of 2.44 Å³ Da^{−1} and a solvent content of 49.2%. The crystals of TtMgtE_{1–275} in the absence of Mg²⁺ diffracted to 3.5 Å resolution (Fig. 2*b*) and belonged to space group *P*2₁2₁2₁ (unit-cell parameters *a* = 77.0, *b* = 100.3, *c* = 100.3 Å), with the asymmetric unit being likely to contain two molecules with an NCS axis. This is consistent with a Matthews coefficient of 3.10 Å³ Da^{−1} and a solvent content of 60.0%. Dimer formation by TtMgtE_{1–275} is apparently inconsistent with the results of size-exclusion chromatography, suggesting that the TtMgtE_{1–275} dimer has an elongated rather than a globular shape. The distinct crystal forms in the presence and absence of Mg²⁺ might indicate an Mg²⁺-dependent conformational change of the MgtE cytosolic domain. The data-collection statistics are summarized in Table 1. The phases have recently been determined by the multiple anomalous dispersion method using crystals of the SeMet-substituted protein.

We thank the beamline staff at BL41XU of SPring-8 (Harima, Japan) for technical help during data collection and Tomoya Tsukazaki for helpful suggestions. This work was supported by a PRESTO Program grant from JST (Japan Science and Technology) to ON, by grants from MEXT to ON and SF and by grants from the Society for Research on Umami Taste, the Danone Institute and the Yamazaki Foundation to ON.

References

- Bennetts, B., Rychkov, G. Y., Ng, H. L., Morton, C. J., Stapleton, D., Parker, M. W. & Cromer, B. A. (2005). *J. Biol. Chem.* **280**, 32452–32458.

- Biemans-Oldehinkel, E., Mahmood, N. A. & Poolman, B. (2006). *Proc. Natl Acad. Sci. USA*, **103**, 10624–10629.
- Bui, D. M., Gregan, J., Jarosch, E., Ragnini, A. & Schweyen, R. J. (1999). *J. Biol. Chem.* **274**, 20438–20443.
- Eshaghi, S., Niegowski, D., Kohl, A., Martinez Molina, D., Lesley, S. A. & Nordlund, P. (2006). *Science*, **313**, 354–357.
- Hmiel, S. P., Snavely, M. D., Miller, C. G. & Maguire, M. E. (1986). *J. Bacteriol.* **168**, 1444–1450.
- Ignoul, S. & Eggermont, J. (2005). *Am. J. Physiol. Cell Physiol.* **289**, C1369–C1378.
- Kehres, D. G. & Maguire, M. E. (2002). *Biomaterials*, **15**, 261–270.
- Lunin, V. V., Dobrovetsky, E., Khutorskaya, G., Zhang, R., Joachimiak, A., Doyle, D. A., Bochkarev, A., Maguire, M. E., Edwards, A. M. & Koth, C. M. (2006). *Nature (London)*, **440**, 833–837.
- Maguire, M. E. (1992). *J. Bioenerg. Biomembr.* **24**, 319–328.
- Maguire, M. E. (2006a). *Curr. Opin. Struct. Biol.* **16**, 432–438.
- Maguire, M. E. (2006b). *Front Biosci.* **11**, 3149–3163.
- Nelson, D. L. & Kennedy, E. P. (1971). *J. Biol. Chem.* **246**, 3042–3049.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Smith, R. L. & Maguire, M. E. (1998). *Mol. Microbiol.* **28**, 217–226.
- Smith, R. L., Thompson, L. J. & Maguire, M. E. (1995). *J. Bacteriol.* **177**, 1233–1238.
- Townsend, D. E., Esenwine, A. J., George, J. III, Bross, D., Maguire, M. E. & Smith, R. L. (1995). *J. Bacteriol.* **177**, 5350–5354.
- Toyoshima, C. & Nomura, H. (2002). *Nature (London)*, **418**, 605–611.
- Wabakken, T., Rian, E., Kveine, M. & Aasheim, H. C. (2003). *Biochem. Biophys. Res. Commun.* **306**, 718–724.