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Structural studies on cytosolic domain of Magnesium transporter MgtE from *Enterococcus faecalis*

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Introduction

Magnesium (Mg^{2+}) is an essential element for growth and maintenance of living cells. It acts as a cofactor for many enzymes and is also essential for stability of the plasma membrane. There are two distinct classes of magnesium transporters identified in bacteria that convey Mg^{2+} from periplasm to cytoplasm (Moncrief and Maguire 1999; Lunin, Dobrovetsky et al. 2006) [ATPase dependent (MgtA and MgtB) and constitutively active (CorA and MgtE)]. Previously published work on Mg^{2+} transporters yielded structures of full length MgtE from *Thermus thermophilus*, determined at 3.5Å resolution, and its cytoplasmic domain with and without bound Mg^{2+} , determined at 2.3 and 3.9Å resolution, respectively (Hattori, Tanaka et al. 2007). Here we report the crystal structure of the Mg^{2+} bound form of the cytosolic portion of MgtE (residues 6-262) from *Enterococcus faecalis* at 2.2Å resolution. The present structure and magnesium bound cytosolic domain structure from *Thermus thermophilus* (PDB ID: 2YVY) are structurally similar. Three magnesium binding sites are common to both MgtE full length and the present structure. Our work revealed an additional Mg^{2+} binding site in the *E. faecalis* structure. In this report, we discuss the functional significance of Mg^{2+} binding sites in the cytosolic domains of MgtE transporters.

Methods

Gene cloning, expression and purification

The target gene for MgtE was cloned from *Enterococcus faecalis* genomic DNA (ATCC 700802D) using forward primer AATGAAGGCCAAGAAATGGAAG and reverse primer ATTTAGAAGCGGCTTTTAAAGGG in our pSGX4(BS) vector. Expression and purification were carried out as described in PepcDB (<http://pepcdb.pdb.org/>) for targetID "NYSGXRC-10001b". The protein consisted of a SerLeu N-terminal cloning artifact following Smt3 cleavage by ULP1 protease followed by residues 2-285 (based on Uniprot Q830V1_ENTFA).

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Crystallization and data collection—Diffraction-quality crystals of seleno-methionine protein were obtained at room temperature *via* sitting drop vapor diffusion against a reservoir solution containing 25%(w/v) PEG3350, 0.2M MgCl₂, 0.1M HEPES (pH 7.5) (1μl of reservoir solution plus 1μl of protein solution at 10mg/ml). Crystals were frozen by direct immersion in liquid nitrogen using the mother liquor plus 15% (v/v) glycerol. Crystals were obtained in the orthorhombic space group P2₁2₁2₁ with two molecules per asymmetric unit. X-ray diffraction data (2.2Å resolution) were recorded under standard cryogenic conditions at X12C Beamline, National Synchrotron Light Source, Brookhaven National Laboratory. Data were reduced and scaled using HKL2000. Data collection and refinement statistics are given in Table 1.

Structure determination—The structure of the cytosolic portion of MgtE (residues 6-262) was determined at 2.2Å resolution by the Single-wavelength Anomalous Dispersion method (SAD). SHELXD yielded positions for all 11 possible Se atoms/monomer using Selenium peak ($\lambda=0.9795\text{\AA}$) diffraction data (Schneider and Sheldrick 2002). Phase refinement with SHARP followed by density modification with SOLOMON yielded an excellent electron density map (Collaborative Computational Project 1994; Fortelle and Bricogne 1997). ARP/wARP was used for automatic model building and was completed using O (Jones, Zou et al. 1991; Morris, Perrakis et al. 2003). The final model was refined using CNS (Brunger, Adams et al. 1998). The refined structure has excellent agreement with the crystallographic data (Table 1). The atomic coordinates (2OUX) are available from the Protein Data Bank.

Cytosolic domain structure—The overall structure contains two near identical monomers, which are related by a non-crystallographic two fold axis (Fig. 1; root-mean-square deviation=0.5Å among equivalent Cα atoms). Each monomer can be conceptually divided into three regions, a regular right handed super α-helical region (residues 6-132), two cystathionine β-synthase (CBS) motif regions (CBS1, residues: 155–194 and CBS2, residues: 219–247) and a connecting α-helical segment (residues 249–262). The super α-helical region has ten α-helices that adopt a tandem arrangement. Each CBS motif contains two anti-parallel β-strands flanked by α-helices on one side. Within the crystallographically-observed dimer, the CBS motifs of the A and B monomers associate in head to head fashion and form a CBS disc 54Å in diameter and 15Å thick, which is sandwiched between the super α-helical regions. Two lines of evidence suggest that the dimer depicted in Fig. 1 corresponds to its solution state. First, analytical gel filtration revealed that *E. faecalis* MgtE exists as a dimer in solution. Second, 1400Å² of solvent accessible surface area is buried on dimer formation. The overall structure is similar to the Mg bound cytosolic domain structure of MgtE from *T. thermophilus*. The rmsd is 2.37Å for 226 equivalent Cα atoms.

The sequence comparison of MgtE full length from *E. faecalis* (NP_816305 residues 1-453) and *T. thermophilus* (YP_144326 residues 7-448) showed 33% sequence identity. The MgtE full length protein structure of *E. faecalis* was modeled using Swiss automated homology modeling server (Fig 2) using full length MgtE from *T. thermophilus* (PDB ID: 2YVX) as a template. As in the case of full length MgtE structure from *T. thermophilus*, the connecting helices in the modeled structure are oriented perpendicular to the membrane interface and interact with transmembrane helices (Fig 2).

Conserved Mg²⁺ ion binding sites within the cytosolic domain—Analyses of residual electron density peak heights and coordination geometries permitted identification of eight Mg²⁺ ions bound to the *E. faecalis* MgtE cytoplasmic domain dimer. Each protomer binds four Mg²⁺ ions, each of which is separated from its nearest Mg²⁺ neighbor by an average distance of 8Å. The four equivalent binding sites in protomers A and B are named

as Mg1, Mg2, Mg3 and Mg4 (Figs. 1, 2, 3 and 4; see Table 2 for a detailed comparison of B-factors and coordination geometries.)

Electronegative cylindrical pores within the cytosolic domain—The MgtE cytoplasmic domain surface diagram shows two quasi-cylindrical pores (length~25Å, diameter~9–15Å) running parallel to the inter-molecular two fold axis of symmetry (Fig. 4). The calculated electrostatic potential surface shows a highly electronegative potential extended throughout the length of the channel. From the top view or perpendicular to membrane plane it appears like a deep electronegative potential valley (Fig. 4). The dense negative charge distribution is due to the lining of negative charge bearing residues like Asp and Glu. The charged residues are concentrated in four specific locations corresponding to the four magnesium binding sites (Mg1, Mg2, Mg3 and Mg4). The residues in the Mg²⁺ binding sites are conserved (between *E. faecalis* and *T. thermophilus*) except for Mg1 site. The Mg1 coordinating residue Glu71 in *E. faecalis* is replaced by a histidine in *T. thermophilus*.

Discussion

Additional conserved putative Mg²⁺ binding site in cytoplasmic domain may also play an ion sensor role

The full length MgtE structure crystallized in the presence of Mg²⁺ ion showed a closed form, where two of its cytosolic connecting helices completely block the extension of the putative membrane ion conduction pore (Hattori, Tanaka et al. 2007). Based on the comparison of Mg²⁺ bound and unbound cytoplasmic domain structures, a movement of the connecting helices was proposed. It is further proposed that this movement of connecting helices leads to the rearrangement of the pore forming transmembrane helices thereby opening the closed ion conduction pore. In their proposed mechanism, the cytosolic MgtE domains play a role of homeostasis sensor by sensing the intercellular concentration of Mg²⁺ ions through their conserved Mg4, Mg5 and Mg6 sites (Hattori, Tanaka et al. 2007).

The additional binding site Mg1 in the cytosolic domain of the present structure shows the metal ion in a fully hydrated form with water molecules mediating the metal coordination. The highly conserved negatively charged residues (Asp/Glu) at Mg1 site further imply that the site is well designed to accommodate cations. While Mg2, Mg3 and Mg4 binding sites are absolutely conserved in other MgtEs, the Mg1 site is less conserved even though it has a cluster of negatively charged residues. So this site could also be a non-specific sink for Mg ions across the whole family. So the Mg1 binding site is also one of the conserved binding pockets for positively charged ions in addition to the Mg2, Mg3 and Mg4 sites that is possibly involved in the sensing of intercellular Mg²⁺ concentration. The unbound Mg²⁺ structure shows that the Mg²⁺ depletion not only disrupts the connecting helices but also unlocks the interaction between the super helical and CBS domains (Hattori, Tanaka et al. 2007). This implies that the additional Mg1 binding site in the present structure saturated with Mg²⁺ ions may also play significant role in holding the CBS and super α -helical domains intact (see Figure 4 in Hattori, Tanaka et al. 2007).

MgtE cytosolic structure may have additional functional and/or mechanistic role

In the Mg²⁺ ions bound or closed conformation state, the dimeric cytosolic structure shows the following structural features. First, the size of the putative cytosolic pore dimensions clearly shows that the pore dimensions are wide enough to allow fully hydrated Mg²⁺ (size ~5Å diameter) ions to pass through it. This is also demonstrated by the lining of four bound Mg²⁺ ions along the cytosolic pores. The second feature is the high electronegative potential along the cytosolic pore. This high negative charge environment is conducive for positively

charged Mg^{2+} ions entering from the membrane to cytoplasmic pore. This positive electrostatic potential is continued up to the Mg4 binding site. Accordingly, it is obvious that the cytosolic pore region up to the Mg4 binding site can act as a negative electrostatic sink for the positively charged Mg^{2+} ions. Beyond the Mg4 site the cytosolic pores are widely open. At the Mg4 site the residues involved in Mg^{2+} binding are highly conserved and the residues interacting with the coordinating water molecules are also partially conserved. This shows that this negative environment up to Mg4 site is maintained throughout the MgtE family of proteins. As proposed in other metal transporters, the four conserved charge dense regions along the putative cytosolic pore may also play a role to concentrate and orient the Mg^{2+} ions towards the cytosolic pores (Yang, Jan et al. 1995; Kuo, Gulbis et al. 2003; Pegan, Arrabit et al. 2005). Because of this characteristic feature, the Mg^{2+} ion bound cytosolic domain seems to play some yet unknown structural and/or mechanistic role (e.g., as a putative cytosolic pore) in addition to the major proposed functional role of ion sensor.

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Figure 1. The top/perpendicular to membrane plane view of MgtE dimer in the crystal lattice. The monomers are shown in blue and orange. The dimer 2-fold axis is almost perpendicular to the page and is indicated with a black ellipse and the connecting helices are shown in green color. The magenta balls represent the bound putative Mg^{2+} ions.

Figure 2. The Swiss automated server modeled structure of MgtE full length from *Enterococcus faecalis* viewed in the plane of the membrane plane. The modeled membrane region is shown in ribbon diagram (gray color) and the cytosolic domain regions are color coded same as figure 1. The Mg^{2+} ions in along the cytosolic pore are shown in magenta color. The membrane surface is indicated.

Figure 3. The Mg^{2+} binding residues at putative Mg^{2+} sites Mg1, Mg2, Mg3 and Mg4 are shown in stick. The putative Mg^{2+} ions are shown in magenta and their hydrated water molecules are in gray balls. The coordination bonds are shown in dotted line. The ribbons color scheme is the same as in Figures 1 and 2.

Figure 4.

Top view of molecular and electrostatic potential surface diagram of cytosolic MgtE dimer. The monomers in the molecular surface diagram are shown in blue and orange as in Figure 1. The bound Mg^{2+} ions are shown in magenta. The range of electrostatic potential is from -1.0 (red) to $+1.0$ (blue) volt. This figure was prepared using CCP4mg (Potterton, McNicholas, et al. 2002).

Table 1

Crystal data and refinement statistics.

Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	a = 77.22 Å α = 90°
	b = 79.66 Å β = 90°
	c = 105.89 Å γ = 90°
Wavelength (Å)	0.9795
Data redundancy	6.5
Resolution (Å)	50 – 2.1
Outer most shell (Å)	2.18–2.10
R _{merge} ¹	0.095(0.52) ²
I/σ(I)	11.4 (5)
Completeness (%)	93 (58)
Refinement statistics	
Resolution (Å)	38–2.16
Number of reflections	32653
R free set	2625
R _w ³ /R _{free}	0.256/0.303
Number of atoms	4130
Average B factor (Å ²)	39.6
Rmsd bond length (Å)	0.006
Rmsd bond angle (°)	1.2
Ramachandran plot	93
Most favored region (%)	
Additional allowed regions (%)	6.9

¹R_{merge} = $\sum_j (|I_h - \langle I_h \rangle|) / \sum I_h$, where $\langle I_h \rangle$ is the average intensity over symmetry equivalents.

²Values within parentheses are for outermost shell

³R-factor = $\sum |F_{obs} - F_{calc}| / \sum |F_{obs}|$

Table 2

Magnesium coordination comparison of cytosolic MgtE domain structures

Cytosolic domain putative Mg ²⁺ binding sites in <i>Enterococcus faecalis</i>				Cytosolic domain putative Mg ²⁺ binding sites in <i>Thermus thermophilus</i>		
Binding sites	Coordinating protein residues	Ave. distance (Å)	B-factors (Å ²)	Binding sites	Coordinating Protein residues	
A [*] B [*]			A [*] B [*]	A [*] B [*]		
Mg1 Mg1	Glu71 (OE1)	2.1	38	Nil	Nil	Nil
Mg2 Mg2	Asp230 (OD1)	2.19	28	Mg5	Mg5	Asp226(OD1)
	Arg227(O1)	2.11				Ala223(O1)
Mg3 Mg3	Asp102 (OD1)	2.2	32	Mg6	Mg6	Asp95(OD1)
	Ala140(O1)	2.1				Gly136(O1)
Mg4 Mg4	Asp98 (OD2)	2.11	28	Mg4	Mg4	Asp91 (OD2)
	Asp251(OD2)	2.17				Asp247(OD2)

* represents the subunit