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Crystallization and preliminary crystallographic studies of CorC, a magnesium-ion transporter

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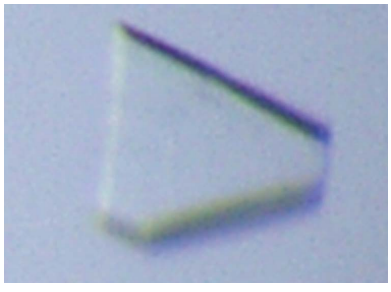

CorC is a magnesium transporter that is involved in the Mg^{2+}-efflux function of the CorA transporter system, an Mg^{2+} channel, from *Shigella flexneri*. Native CorC was purified and crystallized in the native form and in a ligand-free form and diffraction data sets were collected to 2.9 and 3.4 Å resolution, respectively. The native CorC crystals belonged to space group $P2_12_12_1$, with unit-cell parameters a = 64.31, b = 74.44, c = 132.78 Å. The ligand-free CorC crystals belonged to space group $P3_121/P3_221$, with unit-cell parameters $a = b$ = 71.89, c = 125.96 Å. The CorC–ATP complex has also been crystallized and the crystals belonged to space group $P2$ or $P2_1$.

1. Introduction

Magnesium ion (Mg^{2+}) is the most abundant divalent cation in cells, with a concentration of 15–25 m*M* in both prokaryotic and mammalian cells (Maguire & Cowan, 2002; Romani & Scarpa, 1992; Scarpa & Brinley, 1981). It is essential for numerous physiological processes, such as binding to ATP and to enzymes, serving as a crucial structural element for ribosomes, enzymes and membranes and acting as a signal for cell regulation and virulence. Magnesium is unique among divalent cations in that it has the smallest ionic radius and the largest hydrated radius. Therefore, Mg^{2+} transporters and their mechanisms differ from those of other ions. The best characterized Mg^{2+}-transport proteins to date are those from prokaryotic sources, such as CorA and MgtE. Both CorA and MgtE are present in approximately half of currently sequenced microbial genomes. Their eukaryotic homologues have also been found to be involved in Mg^{2+} transport (Schindl *et al.*, 2007; Wabakken *et al.*, 2003). The crystal structures of CorA and MgtE have recently been determined (Hattori *et al.*, 2007; Lunin *et al.*, 2006). In agreement with the unique chemistry of Mg^{2+}, both structures are unique among channels and transporters in terms of topology and symmetry. The structures provide insight into the Mg^{2+}-transport mechanism and suggest that CorA and MgtE might be gated in similar ways through multiple Mg^{2+}-binding sites in the cytosolic domain of the channels.

Interestingly, CorA mediates the influx but not the efflux of Mg^{2+}, whereas MgtE mediates both the influx and the efflux of Mg^{2+} and contributes to magnesium homeostasis. The CorA transporter system contains not only CorA but also CorB, CorC and CorD. The efflux of Mg^{2+} requires the presence of at least one of the CorB, CorC or CorD proteins (Gibson *et al.*, 1991). CorB and CorC are similar to each other in sequence, containing a CBS-domain pair and a HlyC domain. The CBS domain, alternatively called the Bateman domain, is a small domain that was originally identified in cystathionine β-synthase and that has subsequently been found in a wide range of proteins, usually occurring in tandem repeats. It can bind ligands with an adenosyl group such as AMP, ADP, ATP and 5-adenosylmethionine. It has been suggested that CBS domains may play a regulatory role in rendering proteins sensitive to adenosyl-carrying ligands (Kushwaha *et al.*, 2009; Scott *et al.*, 2004). MgtE also contains a cytosolic CBS domain that is involved in magnesium homeostasis. This suggests that the Mg^{2+}-efflux function of CorA and MgtE might be gated in a similar manner through binding of adenine nucleotides by the CBS

domain, as has been observed in AMPK and CLC-5. The HlyC domain is found in many proteins related to ion transport, such as some Na^+/H^+ antiporters, and has been proposed to be involved in modulating the transport of ion substrates. This arrangement of a CBS domain and an HlyC domain is considered to be related to the efflux of Mg^{2+}.

To uncover the role of CorB and CorC in Mg^{2+} efflux, we embarked on crystallographic analysis of CorC. We cloned, expressed and purified CorC and crystallized native CorC, ligand-free CorC and the CorC–ATP complex. Here, we report the crystallization, diffraction data collection and preliminary crystallographic studies of native CorC and ligand-free CorC. The results showed that the crystallization of CorC is obviously affected by adenosines and magnesium ions. This should help us to learn more about the mechanism of magnesium-ion transport and should provide us with a possible regulatory mechanism for the CBS domain.

2. Materials and methods

2.1. Cloning, expression and purification of His-tagged CorC

The *corc* gene was amplified from the *Shigella flexneri* 2a strain 301 genome and cloned into the pET-22b expression vector between *Nde*I and *Xho*I restriction sites. As a result, a six-histidine tag was fused to the C-terminus of the recombinant protein. The recombinant plasmid was transformed into *Escherichia coli* host strain BL21 (DE3) competent cells. The transformed bacteria were grown on an LB plate in the presence of 100 µg ml^{-1} ampicillin overnight at 310 K. A single colony was grown in 50 ml LB medium (all LB medium used contained 100 µg ml^{-1} ampicillin) overnight and 25 ml of the 50 ml bacterial culture was transferred into 1 l fresh LB medium. When the OD_{600} of the culture reached 0.6–0.8, isopropyl β-D-1-thiogalactopyranoside was added to the medium to a final concentration of 1 m*M*. The bacteria were cultured for an additional 4 h and then harvested by centrifugation (Hitachi Himac CRT, R5S2 rotor) at 4000 rev min^{-1} for 30 min. The bacteria were resuspended in 30 ml buffer *A* (300 m*M* NaCl, 10 m*M* imidazole, 50 m*M* phosphate buffer pH 8.0) with 1 m*M* phenylmethanesulfonyl fluoride (PMSF), sonicated for 10 min and centrifuged at 16 000 rev min^{-1} for 30 min (Sigma 3K30, 12150 rotor). The supernatant was applied onto an Ni–NTA column (Novagen) previously equilibrated with buffer *A*. The column was washed with ten column volumes of buffer *B* (300 m*M* NaCl, 20 m*M* imidazole, 50 m*M* phosphate buffer pH 8.0). The protein was eluted with buffer *C* (300 m*M* NaCl, 250 m*M* imidazole, 50 m*M* phosphate buffer pH 8.0). The eluate was further purified on a HiLoad 16/60 Superdex 75 column (Pharmacia) pre-equilibrated with buffer *S* (50 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 5 m*M* EDTA, 5 m*M* dithiothreitol and 5% glycerol). The purified protein was concentrated to 10 mg ml^{-1} and assayed using Dye Reagent Concentrate (Bio-Rad) according to the Lowry method.

During purification, we observed that native purified CorC had possibly bound some nucleotide. To prepare a ligand-free sample of CorC, the protein was purified as described above and then mixed with 2 *M* urea to give a final concentration of 1.8 *M* urea. The solution

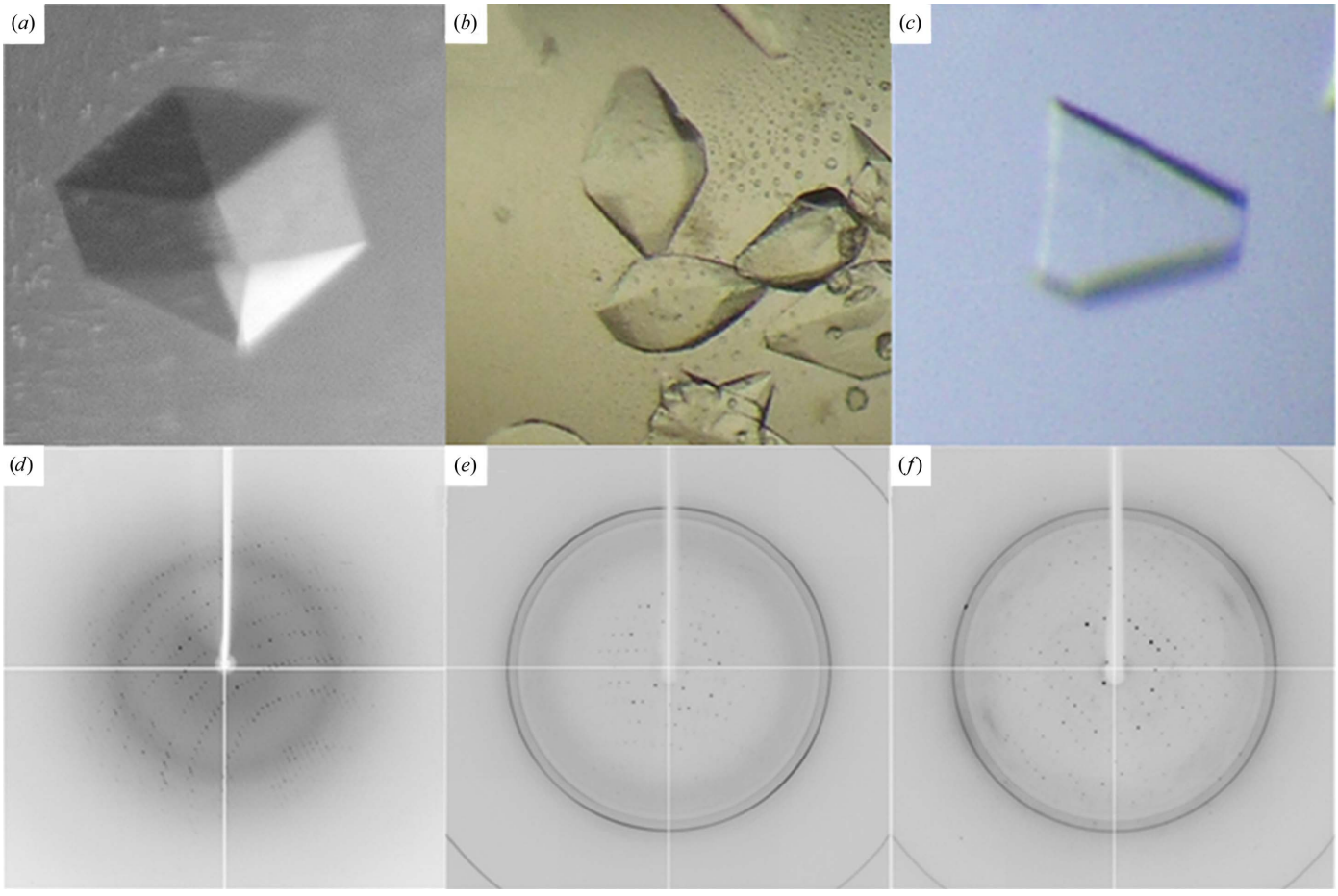

Figure 1
Crystals of native CorC (*a*), ligand-free CorC (*b*) and the CorC–ATP complex (*c*) and their corresponding diffraction patterns (*d*, *e*, *f*).

Table 1
Data statistics for the native CorC, ligand-free CorC and CorC–ATP complex crystals.

The crystal of the CorC–ATP complex showed poor resolution. Values in parentheses are for the highest resolution shell.

	Native CorC	Ligand-free CorC	CorC–ATP
Resolution (Å)	39.16–2.9 (3.06–2.90)	62.99–3.4 (3.58–3.40)	—
Space group	$P2_12_12_1$	$P3_121$ or $P3_221$	$P2$ or $P2_1$
Unit-cell parameters			
a (Å)	64.31	71.89	110.2
b (Å)	74.44	71.89	88.8
c (Å)	132.78	125.96	138.0
α (°)	90	90	90
β (°)	90	90	108.2
γ (°)	90	120	90
No. of unique reflections	14329	9997	—
Completeness (%)	99.8 (99.9)	99.7 (100)	—
Redundancy	4.8 (4.9)	5.4 (5.5)	—
Average $I/\sigma(I)$	13.0 (2.6)	7.19 (2.9)	—
R_{merge} (%)	8.0 (47.5)	6.2 (47.8)	—
$R_{p.i.m.}$ (%)	4.3 (26.4)	3.0 (23.6)	—

was incubated at 277 K for 4 h and loaded onto a MonoQ 5/50GL column (Pharmacia). After washing the column with buffer *E* (2 *M* urea, 5 m*M* EDTA, 50 m*M* Tris–HCl pH 8.0), an NaCl concentration gradient elution was performed; the concentration of NaCl reached 1 *M* in 20 column volumes. The protein was eluted at 200 m*M* NaCl, 5 m*M* EDTA and 50 m*M* Tris–HCl pH 8.0 and was found to be nucleotide-free as judged by UV absorption at 254 nm. The protein was concentrated to 10 mg ml^{-1} and part of the protein solution was mixed with 5 m*M* ATP; it was then used in crystallization.

2.2. Crystallization

The native CorC sample, the CorC–ATP complex sample and the ligand-free CorC sample were used in crystallization trials. Crystallization experiments were set up using the hanging-drop vapour-diffusion method by mixing equal volumes (1 µl) of protein and reservoir solutions and suspending the drop over 400 µl reservoir solution at 293 K. The crystallization condition was optimized by varying the type and concentration of precipitants, salts, buffers and organic compounds and the pH.

2.3. Data collection and processing

A data set was collected from native CorC on BL5A, Photon Factory, KEK, Japan. For cryoprotection, crystals of native CorC were mounted on nylon cryoloops and soaked in reservoir solution containing 10%(*v*/*v*) glycerol for 30 s before freezing them in a liquid-nitrogen stream. A total of 180 frames were collected at a wavelength of 1.00 Å with a crystal-to-detector distance of 200 mm, 1° oscillation and 40 s exposure per frame. A data set was collected from ligand-free CorC on BL17U at SSRF, Shanghai, People's Republic of China. The crystals were mounted on a nylon cryoloop, passed through paraffin oil and then used for diffraction. A total of 180 frames were collected at 1.00 Å wavelength with a crystal-to-detector distance of 200 mm, 1° oscillation and 6 s exposure per frame. *MOSFLM* (v.7.0.4; Leslie, 2006) and *SCALA* (v.6.0) from the *CCP*4 program suite (v.6.0.2; Collaborative Computational Project, Number 4, 1994) were used for indexing, integration and scaling of the diffraction data sets.

3. Results

Crystals of native CorC were obtained using a reservoir solution consisting of 10–20% PEG 3350, 0.2 *M* NaSCN, 10% glycerol pH 6.0–6.6 within 1 d of crystallization setup (Fig. 1*a*). Two crystal forms, trigonal and orthorhombic, were observed in each drop. The trigonal crystals diffracted to low resolution. The orthorhombic crystals diffracted to 2.9 Å resolution (Fig. 1*d*) and belonged to space group $P2_12_12_1$, with unit-cell parameters a = 64.31, b = 74.44, c = 132.78 Å. The asymmetric unit was estimated to contain two native CorC molecules, with a Matthews coefficient of 2.34 Å^3 Da^{-1} and a solvent content of 47.41%. The data-collection statistics are summarized in Table 1.

Crystals of ligand-free CorC were obtained in a condition similar to that used for native CorC and showed the same shape as the trigonal crystals of native CorC (Fig. 1*b*). The ligand-free CorC crystals belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 71.89$, $c = 125.96$ Å, and diffracted to 3.4 Å resolution (Fig. 1*e*). The asymmetric unit was estimated to contain two ligand-free CorC molecules, with a Matthews coefficient of 2.86 Å^3 Da^{-1} and a solvent content of 57.02%. The data-collection statistics are summarized in Table 1.

Crystals of the CorC–ATP complex were obtained with a reservoir solution consisting of 1.86 *M* K_2HPO_4, 0.06 *M* KH_2PO_4 and 5% glycerol 5 d after crystallization setup (Fig. 1*c*). The CorC-ATP crystals were plate-shaped, differing from the thick crystals of native purified CorC and ligand-free CorC, and diffracted to 4 Å resolution (Fig. 1*f*).

We noticed that the native CorC crystallized in both trigonal and orthorhombic forms, whereas the ligand-free CorC only crystallized in a trigonal form; neither of them could be crystallized in conditions similar to those used for the CorC–ATP complex and neither of them could be crystallized in the presence of magnesium ions. These results revealed a difference between the native CorC, the ligand-free CorC and the CorC–ATP complex and suggested that adenine nucleotides and magnesium ions have a great impact on the conformation of CorC. The work reported here has laid the foundations for future structure determination of the CorC protein and for revealing the possible regulatory mechanism of CorC in the CorA system.

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References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Gibson, M. M., Bagga, D. A., Miller, C. G. & Maguire, M. E. (1991). *Mol. Microbiol.* **5**, 2753–2762.
Hattori, M., Tanaka, Y., Fukai, S., Ishitani, R. & Nureki, O. (2007). *Nature (London)*, **448**, 1072–1075.
Kushwaha, H. R., Singh, A. K., Sopory, S. K., Singla-Pareek, S. L. & Pareek, A. (2009). *BMC Genomics*, **10**, 200.
Leslie, A. G. W. (2006). *Acta Cryst.* D**62**, 48–57.
Lunin, V. V., Dobrovetsky, E., Khutoreskaya, 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. & Cowan, J. A. (2002). *Biometals*, **15**, 203–210.
Romani, A. & Scarpa, A. (1992). *Arch. Biochem. Biophys.* **298**, 1–12.
Scarpa, A. & Brinley, F. J. (1981). *Fed. Proc.* **40**, 2646–2652.
Schindl, R., Weghuber, J., Romanin, C. & Schweyen, R. J. (2007). *Biophys. J.* **93**, 3872–3883.
Scott, J. W., Hawley, S. A., Green, K. A., Anis, M., Stewart, G., Scullion, G. A., Norman, D. G. & Hardie, D. G. (2004). *J. Clin. Invest.* **113**, 274–284.
Wabakken, T., Rian, E., Kveine, M. & Aasheim, H. C. (2003). *Biochem. Biophys. Res. Commun.* **306**, 718–724.