BBA - General Subjects 1863 (2019) 25–30

Contents lists available at ScienceDirect

BBA - General Subjects

journal homepage: www.elsevier.com/locate/bbagen

# Loss of cytosolic $Mg^{2+}$ binding sites in the *Thermotoga maritima* CorA $Mg^{2+}$ channel is not sufficient for channel opening

Thomas Kowatz, Michael E. Maguire*

*Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106-4965, USA*

ARTICLE INFO

*Keywords:*
Tm CorA
D89K/D253K
D89R/D253R
Crystal structures, closed, functional
Magnesium
Channel
Transport

ABSTRACT

The CorA $Mg^{2+}$ channel is a homopentamer with five-fold symmetry. Each monomer consists of a large cytoplasmic domain and two transmembrane helices connected via a short periplasmic loop. In the *Thermotoga maritima* CorA crystal structure, a $Mg^{2+}$ is bound between D89 of one monomer and D253 of the adjacent monomer (M1 binding site). Release of $Mg^{2+}$ from these sites has been hypothesized to cause opening of the channel. We generated mutants to disrupt $Mg^{2+}$ interaction with the M1 site. Crystal structures of the D89K/D253K and D89R/D253R mutants, determined to 3.05 and 3.3 Å, respectively, showed no significant structural differences with the wild type structure despite absence of $Mg^{2+}$ at the M1 sites. Both mutants still appear to be in the closed state. All three mutant CorA proteins exhibited transport of $^{63}Ni^{2+}$, indicating functionality. Thus, absence of $Mg^{2+}$ from the M1 sites neither causes channel opening nor prevents function. We also provide evidence that the *T. maritima* CorA is a $Mg^{2+}$ channel and not a $Co^{2+}$ channel.

## 1. Introduction

$Mg^{2+}$ is ubiquitous and plays an important role in many cellular activities. It functions as a cofactor for many enzymes and is necessary for maintenance of genomic stability. Problems in $Mg^{2+}$ homeostasis can cause several diseases [1,2]. Thus, cytosolic concentrations of divalent cations such as $Mg^{2+}$ must be regulated to maintain proper cell health. In about half of bacteria and archaea, the CorA $Mg^{2+}$ channel is primarily responsible for $Mg^{2+}$ homeostasis [3–5]. The eukaryotic homolog of CorA is the Mrs2 $Mg^{2+}$ channel of the mitochondrion and is an essential gene [6]. The CorA of all bacteria tested can also transport $Co^{2+}$ and $Ni^{2+}$ [3,7].

The first X-ray structure of a divalent ion channel was that of CorA from the thermophilic Gram negative marine bacterium *Thermotoga maritima* (Tm CorA) [8]. Tm CorA is a homopentamer with five-fold symmetry about a central pore. Each monomer consists of a large cytoplasmic domain, two transmembrane helices connected via a short periplasmic loop and a very short highly conserved C-terminal tail. The soluble cytoplasmic domain of each monomer consists of seven antiparallel β-strands surrounded by six α-helices. A long seventh helix termed the “stalk helix” kinks upon entering the membrane and becomes the first transmembrane domain (TM1). It contains the highly conserved “YGMNF” motif at the periplasmic end [8–10], which has been proposed to function as a selectivity filter [9,11]. Mutations of residues within that cluster block $Mg^{2+}$ transport [10,12,13]. In most published structures, the periplasmic loop distal to the YGMNF motif is disordered, probably due to its flexibility [8–10].

Crystal structures of Tm CorA show several $Mg^{2+}$ ions bound to the large cytosolic domain. A $Mg^{2+}$ is located between D89 of the α3 helix of one monomer and D253 of the α7 stalk helix of the neighboring monomer in all Tm CorA structures (M1 binding site) [8–10,14]. An additional $Mg^{2+}$ ion interaction within each monomer has been observed in vicinity of L12, E88, D175 and D253 in some structures (M2 binding site) [9,10,14–16].

All published Tm CorA crystal structures are of the closed state of the channel [8–10]. Two possibly open state Tm CorA cryo-EM structures of low resolution (~7 Å) have recently been reported [14]. X-ray crystallography, electrostatic analysis, molecular dynamics simulation and cryo-EM studies have proposed that $Mg^{2+}$ binding to the M1 cytosolic binding sites is responsible for the closed form of CorA [8–10,14–20] and may help keep the N-terminal cytosolic domains together [9]. Mechanistically, it has been proposed that $Mg^{2+}$ dissociation from the M1 site due to low intracellular $Mg^{2+}$ concentration initiates significant conformational changes involving the long stalk helices and the first transmembrane domain leading to a subsequent opening of the pore, allowing $Mg^{2+}$ influx [8–10,14–20]. Conversely,

*Abbreviations:* Tm CorA, Thermotoga maritima CorA; KK-CorA, D89K/D253K Tm CorA; RR-CorA, D89R/D253R Tm CorA

* Corresponding author at: Department of Pharmacology, School of Medicine, Case Western Reserve University, 2109 Adelbert Road, Cleveland, OH 44106-4965, USA.

*E-mail address:* mem6@case.edu (M.E. Maguire).

https://doi.org/10.1016/j.bbagen.2018.09.001
Received 24 January 2018; Received in revised form 29 August 2018; Accepted 3 September 2018
Available online 05 September 2018

as intracellular $Mg^{2+}$ increases, the five M1 sites are proposed to fill, closing the channel.

In this study, we generated both the D253K Tm CorA as well as double mutants by changing both D89 and D253 to either K or R, respectively (denoted KK-CorA and RR-CorA) to disrupt the M1 $Mg^{2+}$ binding site. The KK-CorA and RR-CorA mutants were expressed, purified, and crystallized. Their structures were solved to 3.05 and 3.3 Å resolution, respectively. Neither the KK-CorA nor the RR-CorA crystal structures have $Mg^{2+}$ ions bound to the M1 site.

Further, no major conformational changes of the cytosolic domains, stalk helices and first transmembrane domains could be observed in either structure. Both mutants represent a closed form of the channel. Moreover, the D253K Tm CorA, KK-CorA and RR-CorA channels are able to transport $^{63}Ni^{2+}$, indicating that they are functional channels. Our results indicate that loss of $Mg^{2+}$ interaction at the M1 cytosolic binding site does not cause significant structural conformational changes in the Tm CorA structure and does not prevent channel function. Thus, the absence of $Mg^{2+}$ at the M1 site does not automatically cause channel opening nor is $Mg^{2+}$ binding to the M1 site required to close an open channel.

## 2. Results

### 2.1. Overall structures

Both KK-CorA and RR-CorA structures share the same overall fold as the wild type structures (Figs. 1, 2, and Supplemental Fig. 1). In both mutant structures one pentamer is present in each asymmetric unit. Superposition of KK-CorA and wild type (PDB ID 2IUB) yields an rmsd of 1.0 Å (Fig. 1). When RR-CorA and wild type (PDB ID 4I0U) are superimposed, the rmsd value is 1.4 Å (Fig. 2). Superposition of the mutant structures with each other gives an rmsd of 0.9 Å (Supplemental Fig. 1). Superposition of either structure with other published wild type Tm CorA structures yielded similar results. Superposition of only the stalk helices, the transmembrane domains and C-termini of KK-CorA and RR-CorA with the wild type gives rmsd values of 0.7 Å and 0.8 Å, respectively. Aligning the same structural parts of KK-CorA and RR-CorA to each other yields an rmsd of 0.5 Å. Clearly, the loss of the M1 site in these mutants causes no significant structural changes in the stalk helix and membrane domain. Further, manual inspection of the entire superimposed structures revealed no significant differences. Thus, the mutant Tm CorA proteins displayed essentially the same structure as the wild type (Figs. 1, 2, and Supplemental Fig. 1).

In RR-CorA but not the KK-CorA structure, the periplasmic loop is resolved. There are only small conformational differences with the wild type periplasmic loop (PDB ID 4I0U). Superposition of these regions of RR-CorA, with a recently published Tm CorA cryo EM structure in its closed state (PDB ID 3JCF) also shows some small differences.

Such conformational differences are not surprising as this part of the channel is very flexible [8–10,14,15].

In both mutant structures there are some minor shifts of initial residues of the stalk helices of two monomers away from the permeation tunnel (chains A and B in KK-CorA and B and C in RR-CorA). The main chain carbons (CAs) of F251 of chains B and C of RR-CorA and wild type are located 5.1 and 5.6 Å from each other, respectively (Fig. 3). Distances between main chains of the same residue of subunits A and B are 4.0 and 4.6 Å, respectively, when KK-CorA and wild type are aligned to one another (Supplemental Fig. 2). However, these small movements do not propagate further to the stalk helices or the TM1 pore forming domains (Fig. 3 and Supplemental Figs. 2 and 3).

### 2.2. $Mg^{2+}$ binding at the M1 and M2 sites in KK-CorA and RR-CorA

The mutated M1 site residues K89 and K253 as well as M2 site amino acids (E88, D175) are well resolved in some of the subunits of the KK-CorA structure (Fig. 4(A) and Supplemental Fig. 4). There were no $Mg^{2+}$ ions bound at the M1 sites. In RR-CorA, R89 and D175 side chains are well resolved in most monomers, whereas E88 and R253 side chains are disordered in all five subunits (Fig. 4(B) and Supplemental Fig. 5). There is likewise no electron density for $Mg^{2+}$ in any of the five M1 sites either. In the RR-CorA structure, three of the five M2 sites are occupied with a $Mg^{2+}$ ion (Supplemental Fig. 5). B factors for those divalent cations range from 105 to 116. In KK-CorA only one $Mg^{2+}$ could be modelled into an M2 site (Supplemental Fig. 4, B factor 103).

Alignment of M1 and M2 site residues of KK-CorA and RR-CorA with the wild type structure shows that all of those residues, except the side chains of E88, are at the same position in all three structures disregarding the mutations (Supplemental Fig. 6). In KK-CorA, the side chain of E88 has moved 2 to 3 Å towards the initial N-terminal loop, depending on the subunit. In the case of RR-CorA there is no such shift except in subunit E where a motion of 2.2 Å was observed.

### 2.3. Other $Mg^{2+}$ binding sites in KK-CorA and RR-CorA

In both mutant structures, a $Mg^{2+}$ ion was observed at the pore entry (Supplemental Fig. 4(A) and (B), Supplemental Fig. 5(A) and (B)). A divalent cation occurs at about the same position in other published Tm CorA structures as well [14–16]. It has been proposed that this $Mg^{2+}$ ion could be (partially) hydrated as its location is about 4 Å away from the carbonyl groups of G312 and the side chains of N314 of the YGMNF motif within the pore [15,16]. Similar distances were observed in RR-CorA. In KK-CorA, however, it was not possible to define distances as the YGMNF motif and the loop are somewhat disordered.

Within the cytosolic permeation tunnel, $Mg^{2+}$ ions have been reported near S284 and also near D277 [9,10,14,15]. There is a $Mg^{2+}$ inside the permeation tunnel in both the KK-CorA and the RR-CorA

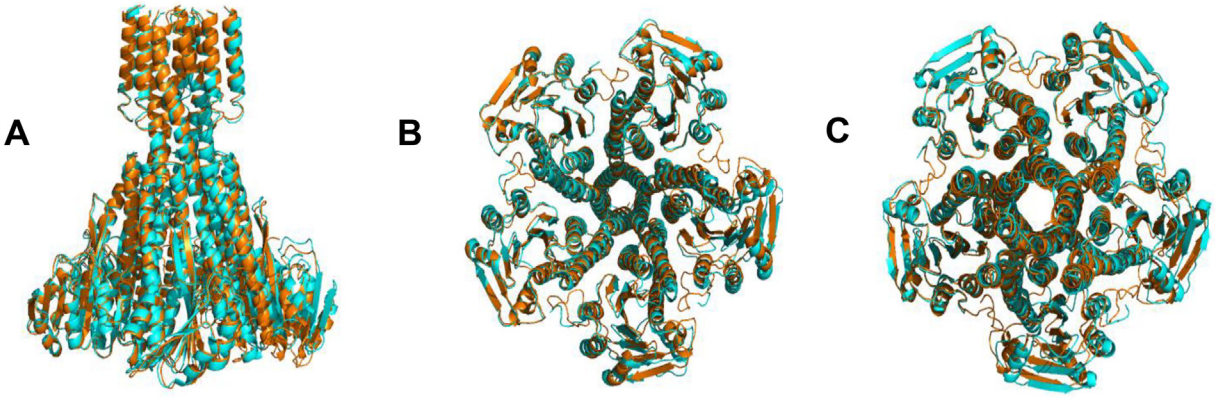


**Fig. 1.** Comparison of KK-CorA with wild type CorA. Cartoon superposition of KK-CorA (cyan) with chains A-E of wild type (orange) Tm CorA pentamer (PDB ID 2IUB). (A) Side view. (B) Cytoplasmic view. (C) Periplasmic view. The rmsd between the two structures is 1.0 Å (1316 to 1316 atoms).