Ligand Binding in the Conserved Interhelical Loop of CorA, a Magnesium Transporter from *Mycobacterium tuberculosis*

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Introduction

CorA is a constitutively expressed Mg^{2+} transporter in most bacteria important for Mg^{2+} uptake. The crystal structures of CorA from *T. maritima* failed to characterize the important conserved interhelical loop in the transmembrane domain. Here, the ion and inhibitor binding properties of the CorA transmembrane domain (CorA-TMD) are uniquely characterized by solution NMR in DPC micelles.

Experimental

The transmembrane domain was expressed and isotopically labeled in *E. coli.* The solution NMR spectroscopy was exclusively performed at the NHMFL on the 600 and 720 MHz NMR spectrometers.

Results and Discussion

Through extensive titration experiments performed on the isolated transmembrane domain of CorA, it was found that two CorA substrates (Mg^{2+} and Co^{2+}) and the CorA specific inhibitor (Hexamine Co(III)) specifically bound to the interhelical loop, sharing the same binding site. Notably, the monovalent cation, Na^{+}, showed no evidence of binding. The shared specificity among these ligands suggests that a native functional binding site for divalent cations has been identified through these studies of the isolated transmembrane domain. In addition, the highly conserved glutamic acid in the "MPEL" motif was shown to directly interact with the ligands. The relatively large dissociation constants indicated weak binding typical of cation channels.

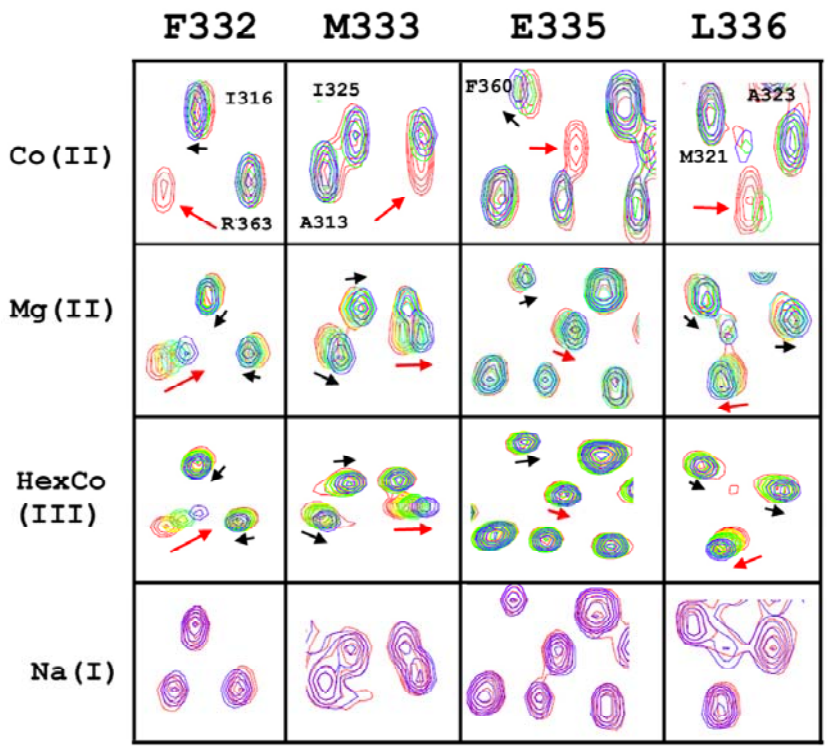

Fig. 1. Binding of CorA ligands on the loop. Regions containing the residues in the loop are selected from the ^{1}H-^{15}N HSQC solution NMR spectra and compared. The concentration of each ligand during titration are: Co (II) (0, 4 and 10 mM), Mg (II) (0, 5, 15, 25, 45, 65 mM), HexCo (III) (0, 5, 10, 20 mM), and Na^{+} (0, 150 mM), respectively. Only the residues that showed significant perturbations are labeled. Arrows indicate the residues sensitive to ligand binding and the direction of the chemical shift changes. The red arrows indicate the residues in the loop, and the black ones indicate the residues in other parts of CorA-TMD. For Co (II), the red arrows just indicate the sensitive residues in the loop.

Conclusions

The present data support the hypothesis that the negative charged loop could act as the initial substrate binding site and then facilitate the transportation by increasing the local substrate concentration.

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