Crystallographic Studies and Cation Selectivity of Cation/Proton Antiporter CpaA

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Abstract

The living environment of bacteria is very extremely changeable in osmotic pressure. In order to adapt to different osmotic conditions, bacteria utilize some channels and transporters regulating intracellular osmotic pressure to protect them from being shrinking or swelling due to the fluctuation of the excellular osmotic pressure. CpaA belongs to the cation/proton antiporter family catalyzing the transport of cation in exchange of protons. The N-terminus of CpaA consists of the transmembrane domain, containing 12 transmembrane helices, which is involved in the exchange of cations for protons. The C terminus of CpaA contains the RCK domain (regulator of conductance of K^+) regulating transport activity of the N-terminal antiporter. Recent studies have shown a novel secondary messenger, cyclic-di-AMP (CDA), specifically binds to CpaA RCK domain and regulates the transport activity. The crystal structure of the CpaA RCK_C domain has been determined previously, revealing one CDA molecule beubg sandwiched by an RCK_C dimer. However, how CDA binding at the RCK domain alters the functionality of the transmembrane domain necessitates further characterization at molecular level. In our study, we aimed to solve the full-length structure of the CpaA RCK domain using experimental phasing and characterize the cation selectivity of CpaA and depict the role of CDA in Cation/Proton exchange using a fluorescence-based assay. We replaced the methionine residues of CpaA RCK with selenomethionine and cocrystallized in the presence of c-di-AMP. We collected the X-ray diffraction data with the highest resolution processed at 2.9 Å and solved the phase angle problem using anomalous dispersion method. The crystal structure of CpaA full-length RCK reveals a dimeric assembly with a Helix-Turn-Helix configuration, and one CDA is located at the RCK_C dimer interface. To assay CDA-regulated Cation/Proton exchange activity, we performed a fluorescence spectroscopic experiment using the pH-sensitive fluorescence dye pyranine, entrapped in proteoliposomes containing CpaA. The Cation influx-induced Proton efflux was monitored by the changes of fluorescence intensity. The assays suggest that CpaA shows the highest activity for the transportation of Na^+ and K^+ but poor for Ca^{2+}, indicating CpaA may prefer transporting monovalent cations to divalent cations. Interestingly, the fluorescence spectroscopic assay shows that CDA binding to CpaA enhanced Na^+ or K^+/H^+ exchange activity. Although we cannot confirm whether CpaA can specifically select sodium or potassium ions in the presense of CDA, it has the function of promoting transport of CpaA. Crosslinking experiments implicated that CDA may help CpaA forming a dimer. This structural and functional studies will enlighten us to comprehend how CDA is involved in the signaling network of ion homeostasis in bacteria.

Keywords- cation/proton antiporter; CpaA; CpaA RCK; c-di-AMP