X-ray Crystallographic and Electron Microscopic Studies of the Potassium Channel KtrAB complex in *Bacillus subtilis*

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Abstract

In bacterial cells, KtrAB complex is critical in the resistance to osmotic stress by mediating uptake of K^+ into the cell. The crystal structure of KtrAB from *Bacillus subtilis* shows that KtrAB complex is composed of a dimeric potassium channel KtrB and a regulating protein KtrA forming an octameric ring on the cytoplasmic side of KtrB. Both structural and biochemical studies suggested that nucleotide-binding, such as ATP and ADP, to the KtrA octamer changes the conformation of the ring, and thus regulates the K^+ flux of KtrB. It has been demonstrated that the KtrA N-terminal domain harbors the binding sites for ATP and ADP. Interestingly, the KtrA C-terminal domain has a specific affinity with c-di-AMP (CDA), a novel second messenger in Gram positive bacteria. In vivo bacterial growth experiments revealed that CDA impedes the K^+ transport activity of KtrB. The crystal structure of CDA-bound KtrA C-terminal domain (CTD) demonstrated the KtrA to CDA stoichiometry of 2:1; however, the structure of full-length KtrA in complex with CDA is still undetermined. Thus, how CDA binding evokes conformational change of KtrA and, subsequently, regulates the transport activity of KtrB, remains elusive. In this study we determined the affinity (Kd) between full-length KtrA and CDA as 4 μM using ITC. We crystallized full-length KtrA in the presence of CDA. The best X-ray diffraction dataset was processed and scaled at resolution up to 2.75 Å. Unfortunately, only two ATPs but no CDA could be identified in our KtrA structure. With differential scanning fluorimetry (DSF) and structural analysis, it is speculated that both ATP and ADP might be the allosteric regulators of CDA, but with different regulatory roles. When ATP binds to the N-terminal domain (NTD) of KtrA, it will inhibit CDA binds to the CTD. However, the binding of ADP will not inhibit CDA binding to the CTD. We currently focus on crystallization trials of CDA-bound KtrA octamer and Cryo-EM screening of KtrAB complex in the presence of ADP and CDA. The structural studies will provide insight into the molecular mechanism of CDA-mediated conformational change of KtrA octameric ring and KtrA-dependent regulation of K^+ conductance of KtrB.

Keywords- KtrAB, c-di-AMP, K channel