Understanding the structural and functional aspects of a novel Mg^{2+}-citrate-binding protein

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Nearly one-third of the proteins require metal ions to accomplish their functions, making them obligatory for the growth and survival of microorganisms in varying environmental niches [1, 2]. In prokaryotes, besides their involvement in various cellular and physiological processes, metal ions stimulate the uptake of citrate molecules. Citrate is a source of carbon and energy and is reported to be transported by secondary transporters. In Gram-positive bacteria, citrate molecules are transported in complex with divalent metal ions, whereas in Gram-negative bacteria, they are translocated by Na^+/citrate symporters (CitS) [3, 4]. Interestingly, the presence of a secondary transporter allowing the translocation of divalent metal ion-complexed citrate in Gram-negative bacteria has not been reported till date. In this study, we report the presence of a novel divalent metal ion-complexed citrate uptake system that belongs to the primary active ABC transporter superfamily. For the uptake, the metal ion-complexed citrate molecules are sequestered by substrate-binding proteins (SBPs) and transferred to transmembrane domains (TMDs) for their transport [1, 2]. Since SBPs are involved in maintaining the selectivity and specificity of the substrate(s) and directionality of the transport, they have been reported to be pivotal. This study reports the crystal structures of an Mg^{2+}-citrate-binding protein (MctA) from a Gram-negative thermophilic bacteria *Thermus thermophilus* HB8 in both apo and holo forms at a resolution range of 1.63 to 2.50 Å. Despite binding various divalent metal ions, MctA follows the coordination geometry to bind its physiological metal ion, Mg^{2+}. The results also suggest a novel subclassification of cluster D SBPs, known to bind and transport divalent metal ion-complexed citrate molecules. Comparative assessment of the open and closed conformations of the wild-type and mutant proteins of MctA suggests a gating mechanism of ligand entry following an "asymmetric domain movement" of the N-terminal domain (NTD) for substrate binding.

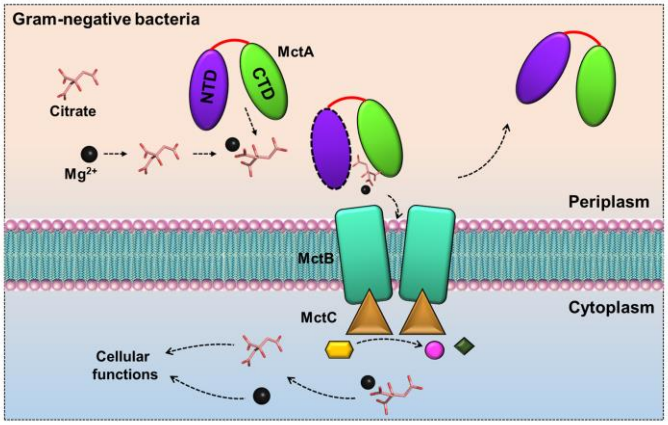

Figure 1. The protein MctA recognizes and binds Mg^{2+}-citrate in the periplasm and transfer it to the MctBC transport system for further translocation of the substrate.

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