ABSTRACT

This research investigates the mechanisms underlying two distinct ABC (ATP-Binding Cassette) importers (i) the type-I glucose-6-phosphate (G6P) importer VCA0625-27 and (ii) the type-II heme importer HutCD-B, in *Vibrio cholerae*, a bacterium that causes cholera. Heme uptake by pathogenic bacteria in the human host is crucial for satisfying their iron needs for essential cellular functions. Despite that, the mechanism of heme import by the ATP-binding-cassette (ABC) transporter HutCD in *Vibrio cholerae* remained unexplored. We have performed biochemical studies on ATPase HutD and its mutants, along with molecular modeling, docking and unbiased all-atom MD simulations on lipid-solvated models of permease-ATPase complex HutCD. The results demonstrated mechanisms of ATP binding/ hydrolysis and trapped transient and global conformational changes in HutCD, necessary for heme internalization. ATPase HutD forms a dimer, independent of the permease HutC. MD simulations demonstrated that a rotational motion of HutC dimer occurs synchronously with the inter-dimeric D-loop interactions of HutDs. F151 of TM4–TM5 loop of HutC, packs with ATP and Y15 of HutD, initiating 'cytoplasmic gate opening' which mimics an 'outward-facing' to 'inward-facing' conformational switching upon ATP hydrolysis. The simulation on 'inward-facing' HutCD culminates to an 'occluded' state. The simulation on heme-docked HutCD indicated that the event of heme release occurs in ATP-free 'inward-facing' state. Gradual conformational changes of the TM5 helices of HutC towards the 'occluded' state facilitate ejection of heme. Through a series of biochemical studies, we have revealed the mode of interactions between HutD and heme and its impact on ATP binding/hydrolysis before its release to cytosol.

Sugar phosphates, on the other hand, are potential sources of carbon and phosphate for bacteria. Despite that the process of internalization of Glucose-6-Phosphate (G6P) through plasma membrane remained elusive in several bacteria. VCA0625-27, made of periplasmic ligand binding protein (PLBP) VCA0625, an atypical monomeric permease VCA0626, and a cytosolic ATPase VCA0627, recently emerged as hexose-6-phosphate uptake system of *Vibrio cholerae*. Here we report high resolution crystal structure of VCA0625 in G6P bound state that largely resembles AfuA of *Actinobacillus pleuropneumoniae*. MD simulations on VCA0625 in apo and G6P bound states unravelled an 'open to close' and swinging bi-lobal motions, which are diminished upon G6P binding. Mutagenesis followed by biochemical assays on VCA0625 underscored that R34 works as gateway to bind G6P. Although VCA0627 binds ATP, it is ATPase deficient in the absence of VCA0625 and VCA0626, which is a signature phenomenon of type-I ABC importer. Further, modeling, docking and systematic sequence analysis allowed us to envisage the existence of similar atypical type-I G6P importer with fused monomeric permease in 27 other gram-negative bacteria.