Structure of ABC Transporter MsbA in Complex with ATP·Vi and Lipopolysaccharide: Implications for Lipid Flipping

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ATP-binding cassette (ABC) transporters are integral membrane proteins critical for the transport of a wide variety of substrate molecules across the cell membrane. MsbA, along with human MDR1 P-glycoprotein, are members of the ABC transporter family that have been implicated in multidrug resistance by coupling ATP binding and hydrolysis to substrate transport. This drug efflux results in resistance to antibiotics in microorganisms and resistance to chemotherapeutic drugs in human cancer cells[1]. Using x-ray diffraction data collected at SSRL beam line 11-1 and ALS, we have determined the 4.2 Å x-ray crystal structure of MsbA in complex with transition state mimic ADP, vanadate (an analog of the γ phosphate of ATP) and the human immunomodulatory substrate Ra lipopolysaccharide. This structure is the first intact ABC transporter in complex with nucleotide and substrate.

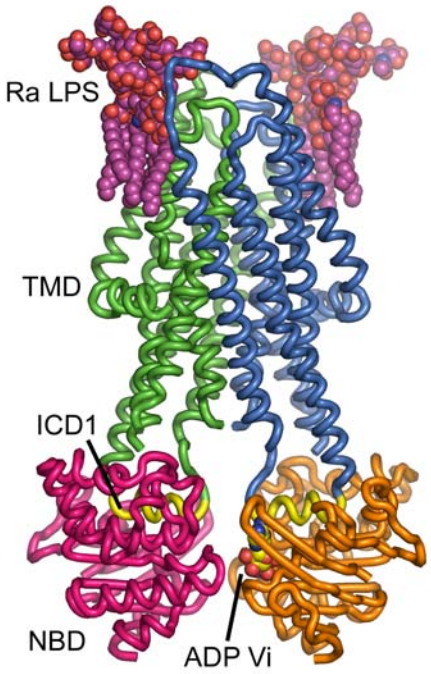

Figure 1. Overall structure of MsbA in complex with ADP, vanadate, Mg^{2+} and Ra lipopolysaccharide (LPS) : TMDs are blue and green, NBDs are pink and orange, ICD1 helix is in yellow, Ra LPS is magenta (oxygen in red, nitrogen in blue), and ADP vanadate is yellow (oxygen in red, nitrogen in blue).

Ubiquitous from bacteria to humans, ATP binding cassette (ABC) transporters are membrane spanning proteins that have a transmembrane domain (TMD) encoding substrate specificity and nucleotide binding domains (NBD) that bind and hydrolyze ATP to drive transport[2, 3]. A subfamily of these transporters functions to export lipids and cytotoxic drugs across the cell membrane. MsbA is a highly conserved gene in Gram-negative bacteria essential for *E. coli* viability and required for phospholipid and lipopolysaccharide (LPS) transport to the outer membrane of bacteria[4-7]. Lipopolysaccharide potently activates the TLR-4 receptor of the mammalian innate immune system in response to bacterial infections and, in high doses, is responsible for septic shock, which is a serious medical condition that can lead to death[8-10]. In addition, MsbA has been shown to share multidrug resistance substrate specificity with LmrA, an ABC transporter from *Lactococcus lactis* that can functionally substitute for human MDR1 P-glycoprotein (PgP) in lung fibroblast cells[11]. Taken together, MsbA represents a vital target to modulate medical complications arising from bacterial infection.

The x-ray structure of MsbA in complex with ADP, vanadate, Mg^{2+} and Ra lipopolysaccharide has revealed the dramatic conformational change in the TMDs that drives substrate transport (Figure 1). Previous structures of MsbA in the absence of nucleotide and substrate, have revealed a cytoplasmic accessible chamber [12, 13]. A large movement of the

TMDs observed in the current structure of MsbA results in a “flipping” of the accessibility of this chamber to the extracellular side of the membrane. Lipopolysaccharide is bound to the membrane-exposed sides of the protein at the dimer interface comprised of TM1, TM5 and TM6 from one monomer and TM2 from the other monomer. In addition, the presence of only a single bound nucleotide was observed in the NBD dimer which is consistent with numerous studies of ABC transporter P-glycoprotein[14] and suggests an alternating catalytic mechanism. The conserved region located between the TMD and NBD, which we collectively call the intracellular domain (ICD) has been suggested to function in coupling the TMD to the NBD. The ICD1 (residues 97-139), is in contact with the NBD and forms a U-like structure consisting of three α-helices. The second helix of ICD1 sits in an elongated groove in the NBD and serves as a conserved pivot about which the NBD could rotate.

The structure suggests a model of substrate “flipping” where the sugar head groups of the lipopolysaccharide molecules are sequestered and then “flipped” in the internal chamber while the hydrophobic tails of the lipid are dragged through the bilayer (Figure 2). While the structure represents an exciting step forward, we look forward to much work ahead to achieve a complete structural mechanistic model of substrate transport by MDR ABC transporters.

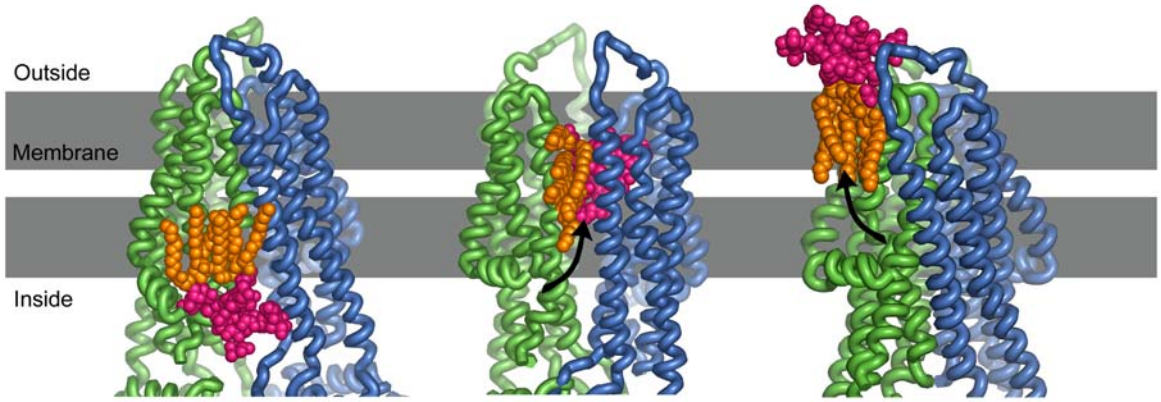

Figure 2. Proposed model for LPS transport by MsbA

(From left to right) LPS initially binds to MsbA in the absence of nucleotide. During substrate binding and ATP hydrolysis, the transporter undergoes conformational changes causing the sugar head group of LPS (pink) to be 'flipped' within the internal chamber of MsbA while the LPS hydrocarbon chains (orange) are dragged through the lipid bilayer. Finally, LPS is presented to the outside of the membrane as observed in this structure.

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