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Snapshots of Endotoxin Extraction from the Gram-negative Inner Membrane

Benjamin Orlando[1], Yanyan Li[2] and Maofu Liao[3]

[1]Michigan State University, East Lansing, Michigan, United States, [2]Westlake University, Boston, Massachusetts, United States, [3]Harvard Medical School, Boston, Massachusetts, United States

Gram-negative bacteria possess a dual membrane architecture, with the outer leaflet of the outer membrane being composed almost entirely of lipopolysaccharide (LPS). LPS is essential for outer membrane formation and antibiotic resistance, and also gives rise to an inflammatory response in humans. LPS biosynthesis begins on the inner leaflet of the outer membrane, followed by transport across the inner membrane, periplasm, and outer membrane. Seven LPS transport proteins A-G (LptA-G) form a complex that spans the inner membrane, periplasm, and outer membrane, and serve to move LPS from the inner to outer membrane. The ATP-binding cassette (ABC) transporter $LptB_2FG$, which is tightly associated with LptC, extracts LPS out of the inner membrane. The mechanism of the entire $LptB_2FGC$ complex and the role of LptC are poorly understood.

Here, we used single-particle cryo-EM to characterize the structures of $LptB_2FG$ and $LptB_2FGC$ in the nucleotide-free and vanadate-trapped states. The transport complexes were incorporated into lipid nanodiscs, which provide a lipid environment to support structural and functional studies. Our cryo-EM structures resolve LPS bound in a central cavity of the transporter, revealing for the first time at high resolution the interaction between LPS and any of the Lpt proteins. The LPS binding pocket in $LptB_2FG$ is composed of alternate hydrophobic and electrostatic regions, providing complementarity to the shape and chemical properties of the bound LPS.

Surprisingly, our structures of $LptB_2FGC$ reveal that LptC inserts its transmembrane helix between the two transmembrane domains of $LptB_2FG$, representing an unprecedented regulatory mechanism for ABC transporters. The LptC transmembrane helix plays a direct role in the binding of LPS, and the nucleotide binding induced conformational changes that mediate LPS export. Our results suggest a role of LptC in coordinating $LptB_2FG$ action and the periplasmic Lpt protein interactions to achieve efficient LPS transport.

Nucleotide trapping with ortho-vanadate provided cryo-EM structures that reveal the conformational rearrangements of $LptB_2FGC$ which serve to extract LPS from the inner membrane. Upon trapping with orthovanadate the LPS binding pocket is completely collapsed, and no bound LPS is observed in the Lpt complex. Furthermore, the transmembrane helix of LptC becomes mobile in the nucleotide bound state, and is no longer tightly associated with the transport complex. Together our nucleotide bound structures demonstrate how binding of ATP initiates large scale conformational changes to extrude LPS to periplasmic components of the Lpt complex. Overall, our structures provide structural snapshots of the Lpt transport complex in different steps of the LPS capture and extraction cycle.

References

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