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Lipopolysaccharide rolls out the barrel

Russell E. Bishop

Department of Biochemistry and Biomedical Sciences, and at the Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario L8S 4K1, Canada

Abstract

Two crystal structures of the LptD–LptE protein complex reveal how the cell-wall component lipopolysaccharide is delivered and inserted into the external leaflet of the bacterial outer membrane.

The inner membrane of many bacteria is surrounded by a protective cell wall, which includes an outer membrane adorned with a tightly packed layer of lipopolysaccharide (LPS) molecules. LPS is an elaborate phospholipid that is extensively decorated with sugars, and its presence provides a barrier against harmful agents such as detergents and lipophilic ('greasy') antibiotics. Many of the cellular processes by which LPS is assembled and transported to the outer membrane are essential for survival, and thus are targets for the development of new antibiotics. In two papers published on *Nature's* website today, Dong *et al.*¹ and Qiao *et al.*² present structural studies that disclose a remarkable molecular mechanism by which LPS arrives at its final destination in the outer membrane.

LPS molecules are mainly assembled by biosynthetic enzymes in the cytoplasm or at the cytoplasmic face of the inner membrane, but the activity of several LPS-modifying enzymes that function at the external face of the inner membrane or in the outer membrane can be used to monitor the molecule on its journey to the cell surface³. First, the region of LPS that anchors it to the membrane, known as lipid A or endotoxin, along with its attached core sugars, is translocated to the external leaflet of the inner membrane. Here, a terminal O-sugar chain can be incorporated before the seven lipopolysaccharide transport proteins (LptA–G) take over (Fig. 1a). An ABC transporter complex (made up of LptF, LptG and two LptB proteins) in the inner membrane detaches LPS from the external leaflet and ushers it along a protein filament (made up of LptA and LptC), which tracks outward into the space between the two membranes⁴. Finally, a complex between LptD and LptE in the outer membrane functions as a station that completes the track for delivering and inserting LPS into the external leaflet of the outer membrane.

Dong *et al.* and Qiao *et al.* present X-ray crystal structures of the complex formed between LptD and LptE from the bacteria *Salmonella typhimurium* and *Shigella flexneri*, respectively. The combined results of the two groups show that LptD is built primarily from structural segments known as β -strands that fold into two domains — a β -jellyroll and a β -barrel. The smaller of the two, the β -jellyroll, extends away from the outer membrane and contains a greasy slot adapted to bind lipid A, while leaving the LPS sugar chain exposed. The same β -jellyroll fold is found in LptA and LptC, which interconnect to align their slots in an extended greasy groove that spirals across the space between the inner and outer

membranes⁵. Thus, it seems that the LptD β -jellyroll is the terminal track for LPS delivery (Fig. 1b).

The structure of this LptD platform is positioned to deliver the LPS directly into the cavity of the much larger domain, the β -barrel, which is composed of 26 membrane-spanning β -strands, the greatest number of these strands ever described for a membrane β -barrel. The LptD β -barrel takes the shape of a kidney bean, with the lobe opposite the β -jellyroll plugged with another outer-membrane protein, LptE. This lipoprotein is positioned almost entirely inside the LptD β -barrel so as to interact most readily with the sugar chain of the delivered LPS — it has only its lipid-modified end anchored outside the β -barrel and within the inner leaflet of the outer membrane. The picture that emerges is one of LptE reorienting the LPS so that it stands vertically, with lipid A positioned transiently inside the open lobe of the β -barrel and the sugar chain protruding out to the cell surface.

Although Dong and colleagues' structure had its β -jellyroll cleaved off and is of slightly lower resolution than that presented by Qiao *et al.*, it is accompanied by functional analyses that confirm an interesting prediction derived from earlier studies of two smaller β -barrel proteins, PagP (refs 6, 7) and FadL (refs 8, 9). The greasy membrane environment drives the formation of strong hydrogen bonds that 'zip' neighbouring β -barrel strands together, but the studies on PagP and FadL have revealed local gaps in β -strand hydrogen bonding that provide gateways for the lateral migration of lipids between their interiors and the external leaflet of the outer membrane.

Could a local gap in β -strand hydrogen bonding similarly support a lateral lipid-migration mechanism in LptD? The region of weakest β -strand hydrogen bonding in the LptD β -barrel is located nearest to the point of LPS passage, between the first and final β -strands (1 and 26, respectively). Dong *et al.* used molecular-dynamics simulations to show that these strands can separate. Furthermore, they observed that bacterial growth was blocked when they judiciously replaced amino acids in each of these β -strands to crosslink them together, supporting the idea that this gap in β -strand hydrogen bonding is necessary for optimal LPS transport. Qiao *et al.* similarly note that β -strands 1 and 2 are distorted in the *S. flexneri* LptD and that local dynamics exist both there and in β -strand 26. However, both structures show the gate in a closed state, and a structure of the complex with bound LPS might be needed to reveal further details of this lateral gating mechanism.

Although it is known that the ABC transporter drives LPS into the LptA–LptC filament¹⁰, it remains to be determined whether additional factors propel LPS out of the LptD–LptE complex so that it inserts into the outer membrane. LPS carries a strong net negative charge, and electrostatic repulsion between neighbouring molecules might help to move them along the filament; bridging of LPS by positively charged magnesium ions in the outer membrane might then facilitate its lateral egress from LptD.

Roughly 200 LptD–LptE complexes are evenly distributed over the surface of a typical bacterium, and each complex transports around 5 LPS molecules to the cell surface every second. A synthetic cyclic-peptide antibiotic is now in phase II clinical trials for treating infections with the bacterium *Pseudomonas aeruginosa* by targeting its LptD and blocking

LPS transport^{11,12}. The structures of the LptD–LptE complexes from *S. typhimurium* and *S. flexneri* thus promise to facilitate the design of better antibiotics, in addition to further clarifying the mechanism by which LPS quite literally rolls out the barrel.

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Figure 1. Structure of the LptD-LptE protein complex

a, The bacterial outer-membrane component lipopolysaccharide (LPS) comprises a lipid A membrane anchor, a core sugar unit and a terminal O-sugar chain. The LPS is assembled in the cytoplasm and exported to the external leaflet of the inner membrane. Seven Lpt proteins (LptA-G) then transport it from the inner to the outer membrane. **b**, Dong *et al.*¹ and Qiao *et al.*² present the crystal structures of the complex formed between LptD and LptE proteins in the outer membrane of *Salmonella typhimurium* and *Shigella flexneri*, respectively, which reveal the route for LPS delivery and insertion into the outer membrane (blue arrow; the *S. flexneri* structure is shown). This structure shows that the sugars of LPS are delivered by the β -jellyroll region of LptD so as to engage first with LptE, which probably reorients the LPS into a vertical position. Insertion of the LPS into the outer-membrane external leaflet then occurs by lateral migration through an opening between the first (1) and final (26) β -strands in the LptD β -barrel.