Structure and mechanism of lipoprotein transport to the cell surface via the lipopolysaccharide translocon in Gram-negative bacteria

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Lipopolysaccharides (LPS) and lipoproteins, two major lipid-containing structural elements of the outer leaflet of the Gram-negative bacterial outer membrane (OM), play critical roles in bacterial cellular physiology and serve as the major disease-causing agents in pathogenic bacteria. Transport of LPS to the cell surface from the periplasm is mediated by the translocon LptDE, yet how lipoproteins reach the cell surface is a long-standing mystery. Here we report the identification of a subset of surface-exposed lipoproteins that interact with LptDE, and the determination of the crystal structures of LptDE from *P. aeruginosa* (paLptDE) and its complex with an endogenous *E. coli* lipoprotein YifL (paLptDE-YifL) at 3.3 Å and 3.0 Å, respectively. The paLptDE-YifL structure snapshots an intermediate state of lipoprotein membrane translocation, revealing that the fatty acyl chains of YifL entered in the OM, whereas its proteinaceous moiety is tucking into the paLptD β barrel lumen. Importantly, YifL is clogged at the paLptD jellyroll-barrel interface, a region that is covalently sealed by the jellyroll-barrel connecting loop on one side and two conserved inter-domain disulfide bonds on the opposite side, suggesting that YifL is only able to be transported to cell surface. In contrast to the jellyroll domain of paLptD in apo-paLptDE, the jellyroll in paLptDE-YifL adopts an overall closed conformation, implying that the whole β-jellyroll bridge over the periplasm may couple the chemical energy derived from cytoplasmic ATP hydrolysis with mechanical force to propel export of lipoprotein via propagated conformational changes along the jellyroll bridge. In addition, we also show that L27-11, a cyclic peptidomimetic antibiotic, may target to the same lipoprotein binding site within paLptD, killing pathogenic *P. aeruginosa* by blocking LPS transport to the cell surface. Taken together, our studies, for the first time, reveal a unique mechanism in detail through which amphipathic lipoproteins and potentially LPS are transported to the cell surface. These evidences also disclose a fact that there exists crosstalk between the Lpt and Lol pathways during OM biogenesis, and extends the classical Lol pathway of lipoprotein trafficking.