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Structure of the Outer Membrane Protein FhuA Presents a Binding Mode for Lipopolysaccharide

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The transport of water-soluble nutrient molecules accross the cell wall into the cytoplasm is an important survival factor for bacteria: roughly 10% of all genes of *E. coli* deal with this process, and a number of transport systems have been devised by nature for this purpose.

To capture iron, a growth-limiting nutrient, bacteria and fungi secrete siderophores (molecular weight, 600–900 Da) like ferrichrome into the external medium. As the concentration of iron-loaded siderophores outside of the bacterial cell is lower than inside, active transport systems in the outer membrane are required for its import into the cell. These are energized by forming a complex with the TonB protein, which is anchored in the periplasmic membrane.

Lipopolysaccharide (LPS, also called endotoxin) is the major component of the outer leaflet of the outer membrane. The innate (also called nonspecific, or nonclonal) immune response in mammals is triggered if LPS is bound by the LPS-binding protein (LBP), which then interacts with membrane-bound CD14 and a Toll-like receptor traversing the membrane of macrophages and monocytes. This primary event leads to the production of radicals and lipid mediators, and to the upregulation of a whole array of cytokines/chemokines. However, in case of a overproduction of these mediators (septic shock), several medical conditions are evoked that are potentially deadly.

We have recently solved and refined at 2.5 Å resolution the X-ray structure (FERGUSON et al. 1998) of one of these active transporters, FhuA, in complex with one molecule of LPS. The major structural building block of FhuA is a β-barrel with 22 antiparallel strands. A "cork" domain formed by residues 1–170 fills the large interior of the barrel. This cork domain exhibits a fold that has so far not been seen in another protein, and completely obstructs the barrel interior. The ferrichrome binding site is at the extracellular surface of the cork, and is mainly formed by aromatic residues from three loops of the cork domain, and by residues from the inner barrel wall.

The LPS molecule is bound to the membrane-exposed outside of the barrel of the protein, with its fatty acid chains in hydrophobic contact with the barrel surface. Its most specific interaction with the protein is electrostatic, and occurs between two phosphates of its lipid A domain and eight positively charged side chains of the extracellular side of the barrel.

To be able to influence the innate immune response by development of antagonistic drugs which can be administered if a patient has a high LPS level in his blood, structural knowledge of the binding mode of LPS to those proteins that bind LPS (and especially LBP) is needed. We hypothesized that some features of the arrangement of positively charged LPS-binding residues on FhuA's barrel surface might not only occur in FhuA, but could be a common motif in LPS-binding proteins (FERGUSON et al. 2000). To test this hypothesis, we used all possible subsets of these eight residues to search for similar arrangements in the Protein Database. The best result was found with one specific subset of four residues.

Inspection of the list of proteins that was returned by the search with these residues shows all those proteins known to interact with LPS, and a number of other mammalian proteins that potentially could also interact with LPS. In the case of those proteins that are known to bind to LPS, the interacting residues obtained from the search have often also been identified by mutational analysis. In all cases, our search procedure confirmed these assignments.

The explanation of this finding is that those proteins, which are involved in the LPS-mediated innate immune response, are dependent upon a pattern recognition mechanism: they recognize lipid A, which is a highly invariant structure among LPS varieties and is capable of inducing a LPS-induced immune response alone.

Based on this identification of residues from LPS-binding proteins, it is possible to model their complexes with LPS, using the FhuA-LPS complex as a template.

References

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