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Characterization of lipopolysaccharide transport protein complex

Research Article

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Abstract: Lipopolysaccharide (LPS) is an essential component of the outer membranes (OM) of most Gram-negative bacteria, which plays a crucial role in protection of the bacteria from toxic compounds and harsh conditions. The LPS is biosynthesized at the cytoplasmic side of inner membrane (IM), and then transported across the aqueous periplasmic compartment and assembled correctly at the outer membrane. This process is accomplished by seven LPS transport proteins (LptA-G), but the transport mechanism remains poorly understood. Here, we present findings by pull down assays in which the periplasmic component LptA interacts with both the IM complex LptBFGC and the OM complex LptDE *in vitro*, but not with complex LptBFG. Using purified Lpt proteins, we have successfully reconstituted the seven transport proteins as a complex *in vitro*. In addition, the LptC may play an essential role in regulating the conformation of LptBFG to secure the lipopolysaccharide from the inner membrane. Our results contribute to the understanding of lipopolysaccharide transport mechanism and will provide a platform to study the detailed mechanism of the LPS transport *in vitro*.

Keywords: *Protein Interactions • Protein complex • Pull down • Protease treatment • Crosslinker*

1. Introduction

The outer membrane of Gram-negative bacteria is a unique asymmetric lipid bilayer, with lipopolysaccharide (LPS) in the outer leaflet and phospholipids in the inner leaflet [1]. The presence of LPS on the cell surface enhances the barrier function of the outer membranes (OM), making many antibiotics used to treat Gram-positive infections ineffective to control Gram-negative pathogens [2]. Although in certain Gram-negative bacteria, the LPS is absent or not necessary [3], it is essential in *Escherichia coli* and most of other Gram-negative bacteria including many pathogens.

LPS is synthesized in the cytoplasmic face of the inner membrane (IM). After synthesis, it is transported to the periplasmic leaflet by MsbA, which is an ATP-binding cassette transporter [4]. Genetic and cellular studies have shown that seven lipopolysaccharide transport (Lpt) proteins, *i.e.* LptA-G, are essential for the following LPS transport. LptB, -F, and -G form an ATP-binding cassette transporter LptBFG. The ATP hydrolysis within LptBFG is thought to provide energy for LPS transport and facilitate the release of mature LPS from the IM and enable its transfer to the periplasmic carrier molecule LptA [5-7]. The LptC anchors to the IM through an N-terminal transmembrane helix to form a complex with LptBFG and its periplasm domain was found to be the essential function region [8]. However, its function is not well understood within the complex LptBFGC [5,9-11]. LptA was proposed to act as a periplasmic chaperone for LPS transport across the periplasm and reported to interact with IM protein LptC [12,13] and OM protein LptD [13]. LptD and LptE form a stable complex at the OM which is proposed to serve as a translocon that facilitates the passage of LPS across the OM bilayer [14]. LptE stabilizes LptD by interacting with its C-terminal domain, and binds LPS, possibly serving as a substrate recognition site at the OM [14].

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