



Characterization of interactions between LPS transport proteins of the Lpt system

Alexandra Bowyer^a, Jason Baardsnes^b, Eunice Ajamian^b, Linhua Zhang^b, Mirosław Cygler^{a,b,*}

^a Department of Biochemistry, McGill University, Montréal, Que., Canada

^b Biotechnology Research Institute, NRC, Montréal, Que., Canada

ARTICLE INFO

Article history:

Received 23 December 2010

Available online 31 December 2010

Keywords:

LPS transport

Lpt system

LptC

LptA

LptE

ABSTRACT

The lipopolysaccharide transport system (Lpt) in Gram-negative bacteria is responsible for transporting lipopolysaccharide (LPS) from the cytoplasmic surface of the inner membrane, where it is assembled, across the inner membrane, periplasm and outer membrane, to the surface where it is then inserted in the outer leaflet of the asymmetric lipid bilayer. The Lpt system consists of seven known LPS transport proteins (LptA–G) spanning from the cytoplasm to the cell surface. We have shown that the periplasmic component, LptA is able to form a stable complex with the inner membrane anchored LptC but does not interact with the outer membrane anchored LptE. This suggests that the LptC component of the LptBFGC complex may act as a dock for LptA, allowing it to bind LPS after it has been assembled at the inner membrane. That no interaction between LptA and LptE has been observed supports the theory that LptA binds LptD in the LptDE homodimeric complex at the outer membrane.

Crown Copyright © 2010 Published by Elsevier Inc. All rights reserved.

1. Introduction

The lipopolysaccharide transport (Lpt) system is present in Gram-negative bacteria, such as *Escherichia coli*, where it is necessary to move lipids across two membrane bilayers to the cell exterior. This double membrane envelops the cytoplasm, protecting it from the external environment and acting as a selectively permeable barrier to compounds passing in and out of the cell. A phospholipid bilayer forms the inner membrane (IM) and an asymmetric bilayer forms the outer membrane (OM), comprising phospholipids within the inner leaflet and mainly outward facing LPS embedded in the outer leaflet. The space between the two membranes contains the periplasm and a thin peptidoglycan layer playing a predominantly structural role [10].

LPS is comprised of three chemical components: a highly conserved and essential lipid A moiety (a glucosamine-based phospholipid) that acts as a hydrophobic membrane anchor, a core oligosaccharide, and a variable length polysaccharide O-antigen chain built from repeating units specific to each bacterial strain [7]. All three components of LPS are synthesized in the cytoplasm. Lipid A is assembled by the Lpx pathway and the addition of core oligosaccharide takes place *in-situ* to form rough-LPS (Ra-LPS) [6,8]. This lipid A-core moiety is translocated from the cytoplasmic leaflet of the IM to the periplasmic leaflet by the ABC (ATP-binding cassette) transporter MsbA [20]. The O-antigen repeating units, each containing 3–5 sugars linked to an undecaprenol moiety,

are preassembled in the cytoplasm and transported across the IM to the periplasm by the flippase Wzx [9]. These repeat units are then sequentially assembled to a growing chain by the polymerase Wzy, and the length of the growing polysaccharide is determined by the polymerase co-polymerase (PCP) Wzz [17]. The complete periplasmic O-antigen chain is attached by the ligase WaaL to the lipid A-core moiety anchored to the outer leaflet of the IM [1], forming smooth-LPS.

The Lpt system contains seven components, the soluble cytoplasmic protein LptB (YhbG), inner membrane LptF (YjgP) and LptG (YjgQ), periplasmic LptC (YrbK) anchored to the IM by a transmembrane helix, soluble periplasmic LptA, periplasmic LptE (RlpB) anchored to the OM by a lipid anchor attached to Cys19 and OM membrane localized LptD (Imp) (Fig. 1). The location of at least one Lpt system component in every cellular compartment points to a coordinated and defined route enabling nascent LPS to traverse the periplasm before being translocated across the OM [15]. Although it has been known for some time that the cytoplasmic protein LptB belongs to the ABC transporter superfamily [13], the function of the IM transmembrane subunits LptF and LptG was only recently identified and they were shown to form the stable complex LptBFG in a subunit ratio of 2:1:1 [11]. This constitutes the four domains of a bacterial ABC transporter: two membrane-spanning domains, each with six transmembrane helices predicted to form an antiparallel heterodimer [5], and two nucleotide binding domains. The absence of any one of Lpt components prevents LPS transport to the OM [14,15,19]. Only LptA [16] and LptC [18] have been structurally characterized.

It has been suggested that the LptBFG complex provides the system with energy from ATP hydrolysis, possibly enabling the

* Corresponding author at: Biotechnology Research Institute, NRC, 6100 Royalmount Avenue, Montréal, Que., Canada H4P 2R2.

E-mail address: mirek@bri.nrc.ca (M. Cygler).

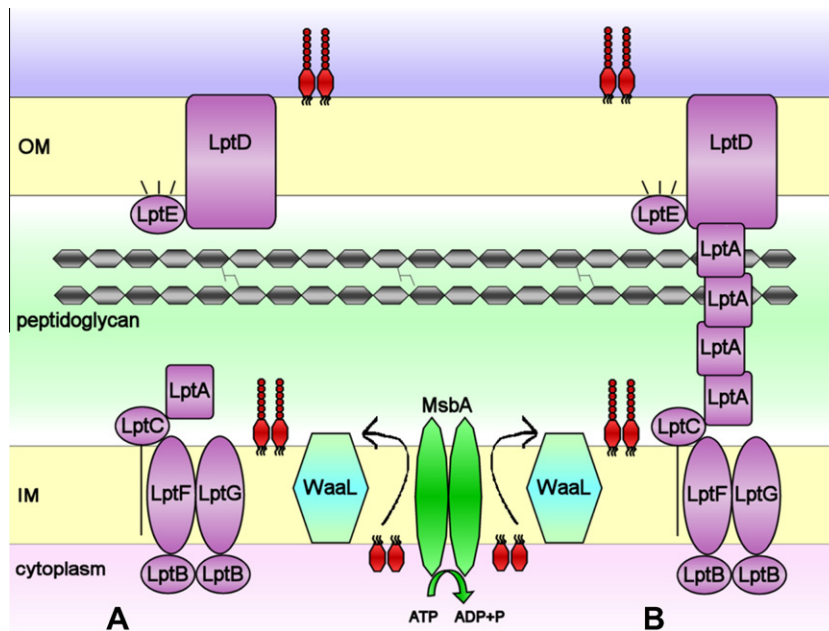


Fig. 1. Two possible models proposed for LPS transport across the periplasm of Gram-negative bacteria. In both cases, MsbA transports the Lipid A-core moiety across the IM and WaaL ligase attaches the completed O-antigen chain. In (A) soluble LptA docks with LptC to pick up the mature LPS and transports it across the periplasm where it docks with LptD and the LPS is received by the LptE component of the LptDE complex before being translocated across the OM. In (B) LptA oligomerizes to form fibrils with a hydrophobic groove running the entire length of the chain into which LPS can bind. This scaffold is the structure that allows the LPS to be transported across the membrane and received by LptE. In both models LptA appears to bind LptC at the IM and LptD at the outer membrane.

mature LPS molecule to be released from the IM and transferred to the periplasmic carrier molecule LptA [16]. The small 19.1 kDa protein LptC anchored to the IM through an N-terminal transmembrane helix forms a complex with LptBFG [11]. Surprisingly, the three-dimensional structure of the periplasmic domain of LptC is very similar to that of LptA [18] despite sharing only 16% sequence identity.

It has been shown that both LptA and LptC can bind LPS *in vitro* and that LptA can displace lipopolysaccharide from LptC [18], consistent with their proposed sequence in this unidirectional transport chain. Once LptA has bound LPS it is unclear how it is transported across the periplasm, but three mechanisms have been considered (Fig. 1). According to one, a soluble LptA monomer or oligomer chaperones the mature LPS molecule across the periplasm and docks at the LptDE complex in the OM [3,12,16,19]. A second mechanism proposes formation of a scaffold consisting of LptA oligomers stacked head-to-tail (fibrils), physically bridging the periplasm with a hydrophobic groove running the entire length of the chain into which LPS could bind [3,12,15,19]. A third mechanism suggests that the LPS transport occurs through membrane contact points, referred to as Bayer junctions [16]. A recent report describing isolation of the trans-envelope complex containing all seven Lpt proteins in a single membrane fraction [3], supports the bridge hypothesis.

By whichever method LptA functions, it is presumed that LPS-bound LptA interacts with a component of the OM LptDE complex to transfer the LPS to the OM. LptE contains a lipid anchor attached to Cys19 but the N-terminus is not required for the interaction between LptD and LptE, and LptE appears to bind the C-terminal transmembrane domain of LptD to form a complex that transports LPS across the OM and inserts it into the outer leaflet [4]. LptE is also a small 19.5 kDa protein that has been shown to be essential for expression of folded LptD *in vitro* and therefore it likely plays a structural role in stabilizing or facilitating folding in LptD [4]. LptE has also been found to bind LPS, so it likely too performs the function of receiving LPS from LptA [4].

Here we present the findings of our investigation into possible interactions between the periplasmic LptA and both the IM anchored LptC and OM anchored LptE.

2. Materials and methods

2.1. Recombinant DNA techniques

The plasmid containing *E. coli* *lptA* gene (UniProt P0ADV1) fused to a non-cleavable C-terminal His₆ tag (LptA-His₆) was kindly provided by Dr. Z. Jia, Queen's University, Ontario [16]. The *E. coli* genes for *lptC* and *lptE* without the predicted signal/TM sequences, corresponding to LptC(26–191) and LptE(20–193), were cloned into modified pET15b vectors with N-terminal His-tagTEV and pRL652 with TEV-cleavable N-terminal GST-tag. The plasmids were transformed into DH5 α cells for amplification and ultimately into BL21(DE3) cells for protein expression. All constructs except GST-LptE could be expressed.

2.2. Overexpression and purification

Glycerol stocks were used to inoculate 10 ml sterile LB media containing 100 μ g/ml ampicillin and grown overnight at 37 °C. 1 L of TB of the same antibiotic strength was inoculated with the starter culture, grown to OD₆₀₀ 1.0, induced with 0.2 mM IPTG and grown overnight at 20 °C. For LptA, the amount of glycerol in TB media was augmented 5-fold [16]. The cells were harvested by centrifugation at 4000 rpm for 30 min. The cell pellet was resuspended to a volume of 50 ml in lysis buffer containing 50 mM TRIS pH 7.8, 5% (v/v) glycerol, 400 mM NaCl, 20 mM imidazole, 0.5 mM benzamidine and 10 μ M leupeptin. The cells were disrupted by sonication on ice for 3 min with 15 s/15 s off, and centrifuged at 15,000 rpm for 45 min to remove cell debris. All constructs showed good expression levels of soluble proteins, typically providing 25–30 mg per 1 L culture.

2.3. Protein purification

The GST-LptC supernatant was loaded onto 2 ml glutathione-sepharose beads pre-equilibrated with lysis buffer, incubated for 30 min at 4 °C and twice washed with lysis buffer supplemented with 1 M NaCl and 500 mM NaCl, respectively. Protein was eluted with a buffer consisting of 50 mM TRIS, pH 7.8, 400 mM NaCl, 5% (v/v) glycerol, and 20 mM reduced glutathione and passed immediately on a desalting column to exchange the buffer into one containing 50 mM TRIS pH 7.8, 150 mM NaCl and 5% (v/v) glycerol. LptA-His₆ and His₈-LptE supernatants were loaded onto 1 ml Ni-NTA beads pre-equilibrated with lysis buffer, incubated for 30 min at 4 °C and twice washed with lysis buffer supplemented first with 1 M NaCl and 20 mM imidazole, then 500 mM NaCl and 40 mM imidazole. Protein was eluted with a buffer consisting of 50 mM TRIS pH 7.8, 400 mM NaCl, 5% (v/v) glycerol, 250 mM imidazole, exchanged to 50 mM TRIS pH 7.8, 150 mM NaCl and 5% (v/v) glycerol on a desalting column.

Mass spectrometry of purified LptA confirmed that the periplasmic signal peptide was correctly processed. Affinity tags from LptC and LptE were removed overnight at 4 °C on appropriate beads with TEV protease:protein ratio of 1:50. To remove the tag and any uncleaved protein the eluted protein was mixed with 0.5 ml of fresh resin, incubated at 4 °C for 1 h with continuous agitation. The eluted tag-less protein was highly pure based on the SDS-PAGE.

2.4. Pull-down assays

To investigate LptA-LptC interaction, 8 mg of GST-LptC was bound to 2 ml glutathione-sepharose beads, washed with 10 times column volume of lysis buffer, loaded with 8 mg of LptA-His₆ and incubated for 1 h at 4 °C with constant agitation. The beads were washed again with 10 column volumes of lysis buffer and the bound proteins eluted with buffer containing 50 mM TRIS pH 7.8, 150 mM NaCl, 5% (v/v) glycerol and 20 mM reduced glutathione. The reverse experiment was carried out with 8 mg of LptA-His₆ bound to 1 ml Ni-NTA beads, washed and 8 mg of GST-LptC added for 1 h at 4 °C, washed again and the proteins eluted with a buffer containing 250 mM imidazole. The presence of both proteins was verified in each case by SDS-PAGE (12% tricine gel). The complex was observed also when untagged LptC was used.

To investigate LptA-LptE interaction, 9 mg of purified LptA-His₆ was bound to 1 ml Ni-NTA beads, washed with 10 column volumes of buffer, beads loaded with 9 mg of untagged LptE, incubated for 1 h at 4 °C with agitation, beads washed and proteins eluted with buffer containing 250 mM imidazole. Only LptA was found in the elution indicating no binding of LptE.

2.5. Size-exclusion chromatography

Samples of both the individual proteins at 15 mg/ml and the LptA-LptC complex at 12 mg/ml were loaded on a Superdex 75 size exclusion column pre-equilibrated with buffer (50 mM TRIS pH 7.8, 150 mM NaCl, 5% (v/v) glycerol). Peak-containing fractions were analyzed by SDS-PAGE. The column was calibrated with appropriate standard proteins (Sigma 12,400–66,000 Da) and apparent molecular weights assigned to each peak.

2.6. Surface plasmon resonance analysis

The affinity of the LptA-His₆-LptC interaction was determined using surface plasmon resonance (SPR). The running buffer contained 10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA and 0.05% Tween 20. The measurements were carried on a ProteOn™ instrument (BioRad). Approximately 600 resonance units (RUs) of

a 545 nM solution of LptA-His₆ was captured at a flow rate of 25 μl/min for 360 s onto a 5000 RU anti-His₆ (Abcam Inc.) surface previously prepared in a ligand channel of a GLM sensor chip using standard sNHS/EDC methods following manufacturer's protocol. Immediately after the capture of LptA-His₆, orthogonal analyte injections over the captured ligand surface were carried out. An initial buffer injection for 30 s at a flow rate of 100 μl/s to stabilize the baseline was followed by the simultaneous injection at 50 μl/min for 120 s of five concentrations of LptC (3× serial dilution from 1000 to 12.3 nM) and buffer for double referencing. The affinity constant for the interaction between LptA-His₆ and LptC was determined from the aligned and referenced sensograms using ProteOn Manager v3.2 and both the equilibrium fit model for affinity (K_D) and Langmuir model with drift for kinetics (k_a , $M^{-1}s^{-1}$ and k_d , s^{-1}).

3. Results and discussion

Of the seven components of the Lpt system only the LptA component is present as a soluble protein in the periplasm. Therefore, we investigated whether LptA interacts with LptC and LptE, two small proteins, each having a periplasmic domain anchored to the IM or OM by an N-terminal transmembrane helix or a lipidated residue, respectively. We expressed and purified full-length LptA-His₆ and confirmed by mass spectrometry that its signal peptide was indeed removed, as well as N-terminally tagged LptC and LptE devoid of the TM and signal regions. The formation of stable complexes was followed by pull-downs and size-exclusion chromatography. The verification of protein-protein interactions was also quantified by SPR, which would allow identifying weaker, transient interactions that could have been undetectable by pull-down assays.

3.1. Interaction between LptA and LptC

The pull-down assays between LptA and LptC performed under a variety of conditions (see Section 2) showed interaction between these two proteins. This was observed using GST-LptC as bait (Fig. 2A, lane 4) and also when LptA-His₆ was used as bait (Fig. 2A, lane 7). The LptA remained bound to LptC even after a wash with 1 M NaCl. The presence of the LptA-His₆-LptC complex was also verified by size-exclusion chromatography. The fractions containing both LptA-His₆ and untagged LptC from the pull-down experiments eluted from the Superdex 75 column as a single peak with an apparent molecular weight of 63 kDa (Fig. 2B) suggesting a heterotrimer. As a control, gel filtration of purified LptA-His₆ and untagged LptC was performed individually, as well as the equimolar mixture of LptA-His₆ and LptC. LptC appeared to be a dimer with molecular weight of ~46 kDa. LptA-His₆ eluted in earlier fractions that corresponded to an apparent molecular weight of ~76 kDa, suggesting oligomeric state, either a trimer or a tetramer. The mixture of LptA and LptC eluted as a single peak with an apparent molecular weight of ~61 kDa just like the fractions from the pull-down. The observed contacts between the molecules in the crystals of LptA show that they associate head-to-tail (N-to-C) [16] while LptC molecules associate tail-to-tail (C-to-C) [18]. While the observed LptA association is, in principle, possible in the cell, the observed LptC association is physiologically unlikely since LptC is membrane-anchored by the N-terminal TM, which would extend from the opposite ends of the crystallographic dimer. Thus species with the apparent MW of ~61 kDa is most likely (LptA)₂LptC, in agreement with an LptC monomer present in the LptC-LptBFG complex [11].

The protein oligomeric states of LptA and LptC determined by size-exclusion chromatography differ from those previously stated in the literature [16,18], but were consistently observed in our

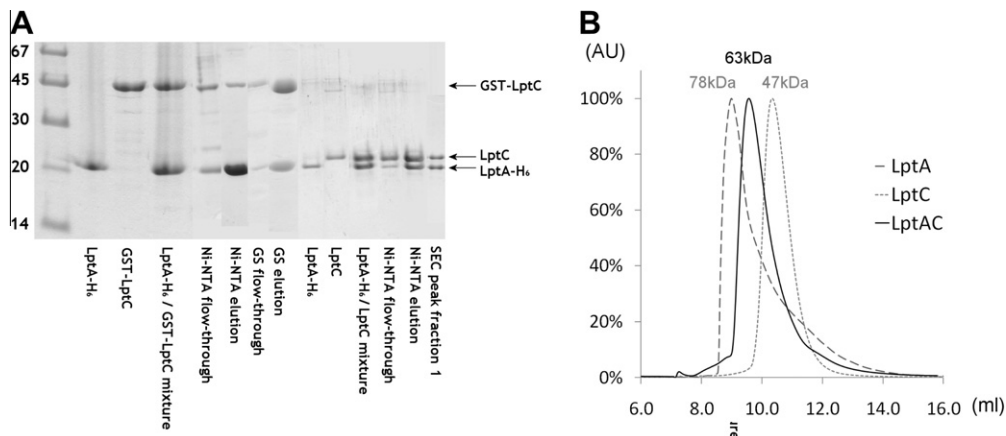


Fig. 2. Complex formation between LptA and LptC as shown by pull-down assays and SEC and visualized on SDS–PAGE tricine gels. (A) Purified LptA-His₆ (lane 2) and GST-LptC (lane 3) were mixed (lane 4) and run on both Ni-NTA and glutathione-sepharose (GS) beads. In both cases, lanes 6 and 8 respectively, a complex was eluted. Purified LptA-His₆ (lane 9) and LptC (lane 10) were also mixed and capture on Ni-NTA beads again showed the presence of a complex in the eluted fractions (lane 13). The eluted LptA-His₆–LptC complex was analyzed by SEC and the single eluted peak contained the complex (lanes 14). (B) SEC of LptA-His₆ indicated a molecular weight of 78 kDa, corresponding to a tetramer, and LptC indicated a molecular weight of 47 kDa, corresponding to a dimer. SEC of the LptA-His₆–LptC complex showed a single peak with an indicated molecular weight of 63 kDa, corresponding to a trimer.

experiments. To establish that these observations were not the result of experimental variation, purification of both proteins was repeated in buffers containing 20 mM sodium acetate pH 5.5 instead of Tris, and also following the purification protocols detailed in the individual crystallization papers for LptA [16] and LptC [18]. Under none of these purification conditions were monomers of either LptA or LptC observed. The observation of LptA as an oligomer, not a monomer, could be reconciled with the head-to-tail binding of LptA molecules in the crystal and would indeed be for the LptA fibril model. Even in the shuttle model, a larger oligomer of LptA is not excluded and may in fact allow more extensive binding of the LPS molecule to provide support for this inherently flexible molecule during transport.

The proteins used here are correctly folded as both LptA-His₆ and LptC (untagged) have been successfully crystallized, when purified in the 20 mM sodium acetate pH 5.5 buffers, and the resultant structures are very similar to those previously published [16,18]. LptA molecules pack into long fibrils (PDB code: 2R19) [16]. The reported crystal structure for LptC (PDB code: 3MY2) forms a lattice of dimers, illustrating that the molecules are indeed capable of binding along their C-termini to form homodimers; therefore crystal structure provides support for existence of dimers in solution, as we consistently observed both with His₆-tagged and untagged protein. The observed dimer is not expected to be biologically relevant *in vivo* as each LptC monomer is anchored to the outer surface of the IM, physically preventing dimerization in the manner observed in the crystal structure.

SPR experiments were used to qualitatively assess the interaction between LptA and LptC. The LptA-His₆ protein was bound to the anti-His₆ surface of the SPR chip but showed moderate dissociation (Fig. 4A). In combination with the fast binding kinetics of the LptA–LptC interaction, the determination of the kinetic rate constants for this interaction has a large standard deviation error. The best model to account for this experimental situation is a Langmuir binding model with drift. The derived K_D value was 500 ± 200 nM, based on the ratio of the rate constants ($k_d s^{-1} / k_a M^{-1} s^{-1}$). A more reliable way to interpret the data for the interaction with fast kinetics is analysis of the plateau response from the binding isotherms, which is independent of the kinetics. The K_D determined by this approach, 480 ± 60 nM (Fig. 4B), correlates very well with the value determined using the kinetic data. We have also attempted to bind LptA covalently to the surface of the

sensor chip. The kinetics for covalently bound LptA with untagged LptC was similar to described above, with K_D value of 2 μ M. The somewhat larger K_D value could be due to a lower effective concentration of LptA due to a random orientation of the molecules on the chip. Importantly, both experiments showed an interaction between LptA and LptC.

Our results are in contrast with a recent report where no stable interactions between LptA and LptC could be detected [18]. While we cannot explain this discrepancy, in our focused study we were able to observe the interaction by multiple methods, namely pull-downs, SEC and SPR, and under several experimental conditions.

3.2. LptA does not interact with the periplasmic segment of LptE

Similar experiments performed to detect interactions between LptA and LptE detected no such interaction. The pull-down assay performed with LptA-His₆ and untagged LptE showed that LptE was found only in the flow-through and wash fractions. SDS–PAGE analysis of the imidazole-eluted fractions showed only the presence of LptA (Fig. 3A). Size-exclusion chromatography was also carried on a mixture of LptA and LptE. On its own, LptE showed a single peak (~ 27 kDa). The mixture showed two distinct peaks corresponding to LptA and LptE (Fig. 3B). Similarly, SPR experiment where the untagged LptE was flown across surface-bound LptA showed no binding, eliminating the possibility of even weak interactions between these two proteins.

In summary, our studies demonstrate that LptA interacts with LptC, but not with LptE. Moreover, size-exclusion chromatography suggests a stable complex with apparent (LptA)₂LptC stoichiometry. The SPR shows a fast on-off kinetics suggesting a complex that readily assembles and dissociates.

We propose that the LptC component of the LptBFGC IM complex acts as a dock or an anchor for LptA oligomers to maintain their proximity to mature LPS in the IM and thereby assist with LPS extraction and uptake by the protein. This proposed function of LptC is consistent with recent literature but cannot differentiate between models of the LptA as a soluble shuttle or LptA forming fibrils. In the first case LptC would form a transient and in the second a permanent anchor.

LptE forms a complex with LptD. Sequence analysis indicated that the periplasmic N-terminal domain of LptD belong to the OstA superfamily, along with LptA and LptC [2]. Therefore, we postulate

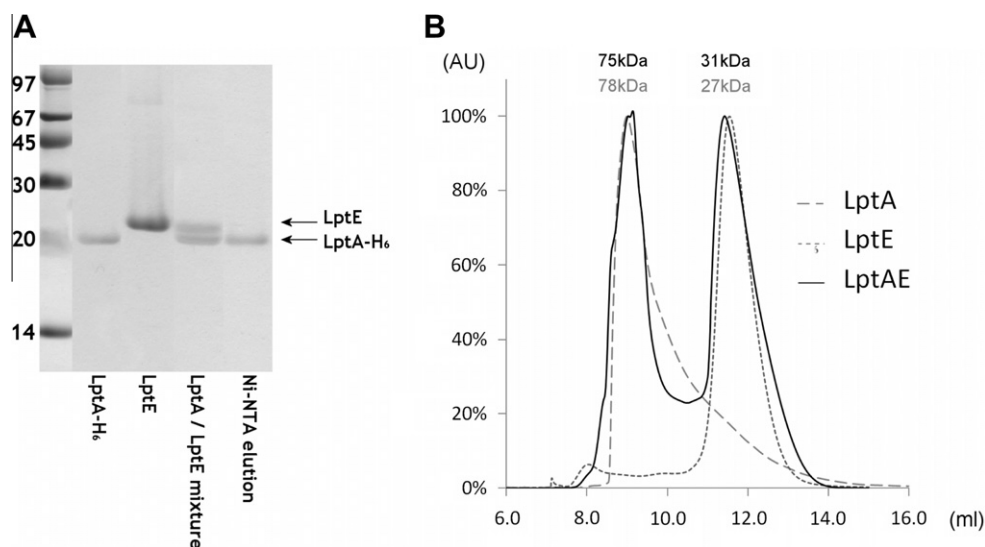


Fig. 3. No complex is observed between LptA and LptE, either by pull-down assays or SEC. (A) Purified LptA-His₆ (lane 2) and LptE (lane 3) were mixed (lane 4) and run on Ni-NTA beads. LptA-His₆ alone was observed in the eluted fractions (lane 5). (B) SEC of LptE indicated a molecular weight of 27 kDa, corresponding to a monomer. SEC of the mixture of LptA-His₆ and LptE complex showed two distinct peaks, corresponding to those of LptA-His₆ and LptE alone.

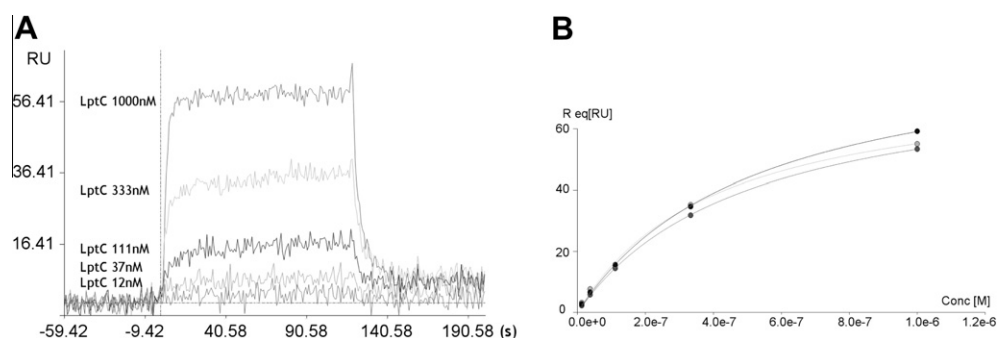


Fig. 4. Surface plasmon resonance experiments revealed an interaction between LptA-His₆ and LptC. (A) LptA-His₆ was indirectly immobilized on the chip surface and five concentrations of untagged LptC, from 1000 to 12.3 nM with a 3× serial dilution, were flowed across. A fast rate of association and dissociation was observed in the binding sensograms, showing that the two proteins do interact. (B) The sensograms fit a Langmuir binding model with drift, giving a K_D value of 480 ± 60 nM derived from the binding isotherms.

that LptA interacts with the N-terminal domain of LptD and that LptE performs a role in stabilizing LptD and in binding LPS, perhaps even receiving it from LptA [3]. According to our data we propose that periplasmic LptA docks to the IM through interactions with LptC and to the OM by interacting with LptD, possibly transferring the mature LPS to LptE but not directly binding to it.

Acknowledgment

This work was supported by CIHR Grant MOP-89787 to M.C.

References

- [1] P.D. Abeyrathne, C. Daniels, K.K. Poon, M.J. Mawehish, J.S. Lam, Functional characterization of WaaL, a ligase associated with linking O-antigen polysaccharide to the core of *Pseudomonas aeruginosa* lipopolysaccharide, *J. Bacteriol.* 187 (2005) 3002–3012.
- [2] M.P. Bos, J. Tommassen, Biogenesis of the Gram-negative bacterial outer membrane, *Curr. Opin. Microbiol.* 7 (2004) 610–616.
- [3] S.S. Chng, L.S. Gronenberg, D. Kahne, Proteins required for lipopolysaccharide assembly in *Escherichia coli* form a transenvelope complex, *Biochemistry* 49 (2010) 4565–4567.
- [4] S.S. Chng, N. Ruiz, G. Chimalakonda, T.J. Silhavy, D. Kahne, Characterization of the two-protein complex in *Escherichia coli* responsible for lipopolysaccharide assembly at the outer membrane, *Proc. Natl. Acad. Sci. USA* 107 (12) (2010) 5363–5368.
- [5] D.O. Daley, M. Rapp, E. Granseth, K. Melen, D. Drew, G. von Heijne, Global topology analysis of the *Escherichia coli* inner membrane proteome, *Science* 308 (2005) 1321–1323.
- [6] W.T. Doerrler, Lipid trafficking to the outer membrane of Gram-negative bacteria, *Mol. Microbiol.* 60 (2006) 542–552.
- [7] F. Hunt, Patterns of LPS synthesis in Gram negative bacteria, *J. Theor. Biol.* 115 (1985) 213–219.
- [8] C.L. Marolda, L.D. Tatar, C. Alaimo, M. Aebi, M.A. Valvano, Interplay of the Wzx translocase and the corresponding polymerase and chain length regulator proteins in the translocation and periplasmic assembly of lipopolysaccharide O antigen, *J. Bacteriol.* 188 (2006) 5124–5135.
- [9] R. Morona, L. Purins, A. Tocilj, A. Matte, M. Cygler, Sequence-structure relationships in polysaccharide co-polymerase (PCP) proteins, *Trends Biochem. Sci.* 34 (2009) 78–84.
- [10] P.F. Muhlrardt, R.G. Jochen, Asymmetrical distribution and artifactual reorientation of lipopolysaccharide in the outer membrane bilayer of *Salmonella typhimurium*, *Eur. J. Biochem.* 51 (1975) 343–352.
- [11] S. Narita, H. Tokuda, Biochemical characterization of an ABC transporter LptBFGC complex required for the outer membrane sorting of lipopolysaccharides, *FEBS Lett.* 583 (2009) 2160–2164.
- [12] N. Ruiz, D. Kahne, T.J. Silhavy, Transport of lipopolysaccharide across the cell envelope: the long road of discovery, *Nat. Rev. Microbiol.* 7 (2009) 677–683.
- [13] P. Sperandio, R. Cescutti, R. Villa, C. Di Benedetto, D. Candia, G. Dehò, A. Polissi, Characterization of lptA and lptB, two essential genes implicated in lipopolysaccharide transport to the outer membrane of *Escherichia coli*, *J. Bacteriol.* 189 (2007) 244–253.
- [14] P. Sperandio, G. Dehò, A. Polissi, The lipopolysaccharide transport system of Gram-negative bacteria, *Biochim. Biophys. Acta* 1791 (2009) 594–602.

- [15] P. Sperandeo, F.K. Lau, A. Carpentieri, C. De Castro, A. Molinaro, G. Dehò, T.J. Silhavy, A. Polissi, Functional analysis of the protein machinery required for transport of lipopolysaccharide to the outer membrane of *Escherichia coli*, *J. Bacteriol.* 190 (2008) 4460–4469.
- [16] M.D.L. Suits, P. Sperandeo, G. Dehò, A. Polissi, Z. Jia, Novel structure of the conserved Gram-negative lipopolysaccharide transport protein A and mutagenesis analysis, *J. Mol. Biol.* 380 (2008) 476–488.
- [17] A. Tocilj, C. Munger, A. Proteau, R. Morona, L. Purins, E. Ajamian, J. Wagner, M. Papadopoulos, B.L. Van Den, J.L. Rubinstein, J. Fethiere, A. Matte, M. Cygler, Bacterial polysaccharide co-polymerases share a common framework for control of polymer length, *Nat. Struct. Mol. Biol.* 15 (2008) 130–138.
- [18] A.X. Tran, C. Dong, C. Whitfield, Structure and functional analysis of LptC, a conserved membrane protein involved in the lipopolysaccharide export pathway in *Escherichia coli*, *J. Biol. Chem.* 285 (2010) 33529–33539.
- [19] A.X. Tran, M.S. Trent, C. Whitfield, The LptA protein of *Escherichia coli* is a periplasmic lipid A-binding protein involved in the lipopolysaccharide export pathway, *J. Biol. Chem.* 283 (2008) 20342–20349.
- [20] A. Ward, C.L. Reyes, J. Yu, C.B. Roth, G. Chang, Flexibility in the ABC transporter MsbA: alternating access with a twist, *Proc. Natl. Acad. Sci. USA* 104 (2007) 19005–19010.