MECHANISM OF RECOGNITION OF LPS BY LAPC AND A REQUIREMENT FOR NEW REGULATORS INCLUDING POLYPHOSPHATE KINASE PPK

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A balance between lipopolysaccharide (LPS) and phospholipids, the two key components of outer membrane, requires a regulated control of the amount of the essential LpxC enzyme that mediates the first committed step in LPS biosynthesis. This is achieved in *Escherichia coli* by proteolytic control of LpxC amounts by the FtsH-LapB complex, which is inhibited by the activity of essential inner membrane protein LapC, whose mode of action is not completely understood. *lapC* mutant bacteria expressing only the essential transmembrane domain of LapC exhibit a temperature-sensitive phenotype, which can be overcome by various suppressors, including overproduction of GnsA, whose function remains unknown. The identification of factors that abrogate GnsA-mediated suppression revealed a requirement for PhoU, a protein involved in the regulation of PhoB/R two-component system, polyphosphate kinase and specific processes involved in cell envelope homeostasis. Examination of LPS of *lapC* mutant bacteria revealed preponderance of glycoforms with a third Kdo and rhamnose in the inner core and the lipid A part, exhibiting non-stoichiometric incorporation of the palmitoyl chain. Overexpression of the *gnsA* gene suppressed the incorporation of palmitoyl chain, PhoB/R-mediated GlcUA incorporation into the LPS inner core and increased the ratio of unsaturated vs saturated fatty acids, revealing a role in balancing LPS composition and membrane fluidity. Using mutagenesis, purification of various LapC mutant proteins and isothermal titration calorimetry (ITC), we show that LapC recognizes LPS using its N-terminal anchor TM1 domain, revealing the mechanism of LapC function in the regulated assembly of LPS.

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