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Biosynthesis and structure–activity relationships of the lipid a family of glycolipids

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Lipopolysaccharide (LPS), a glycolipid found in the outer membrane of Gram-negative bacteria, is a potent elicitor of innate immune responses in mammals. A typical LPS molecule is composed of three different structural domains: a polysaccharide called the *O*-antigen, a core oligosaccharide, and Lipid A. Lipid A is the amphipathic glycolipid moiety of LPS. It stimulates the immune system by tightly binding to Toll-like receptor 4. More recently, Lipid A has also been shown to activate intracellular caspase-4 and caspase-5. An impressive diversity is observed in Lipid A structures from different Gram-negative bacteria, and it is well established that subtle changes in chemical structure can result in dramatically different immune activities. For example, Lipid A from *Escherichia coli* is highly toxic to humans, whereas a biosynthetic precursor called Lipid IV_A blocks this toxic activity, and monophosphoryl Lipid A from *Salmonella minnesota* is a vaccine adjuvant. Thus, an understanding of structure–activity relationships in this glycolipid family could be used to design useful immunomodulatory agents. Here we review the biosynthesis, modification, and structure–activity relationships of Lipid A.

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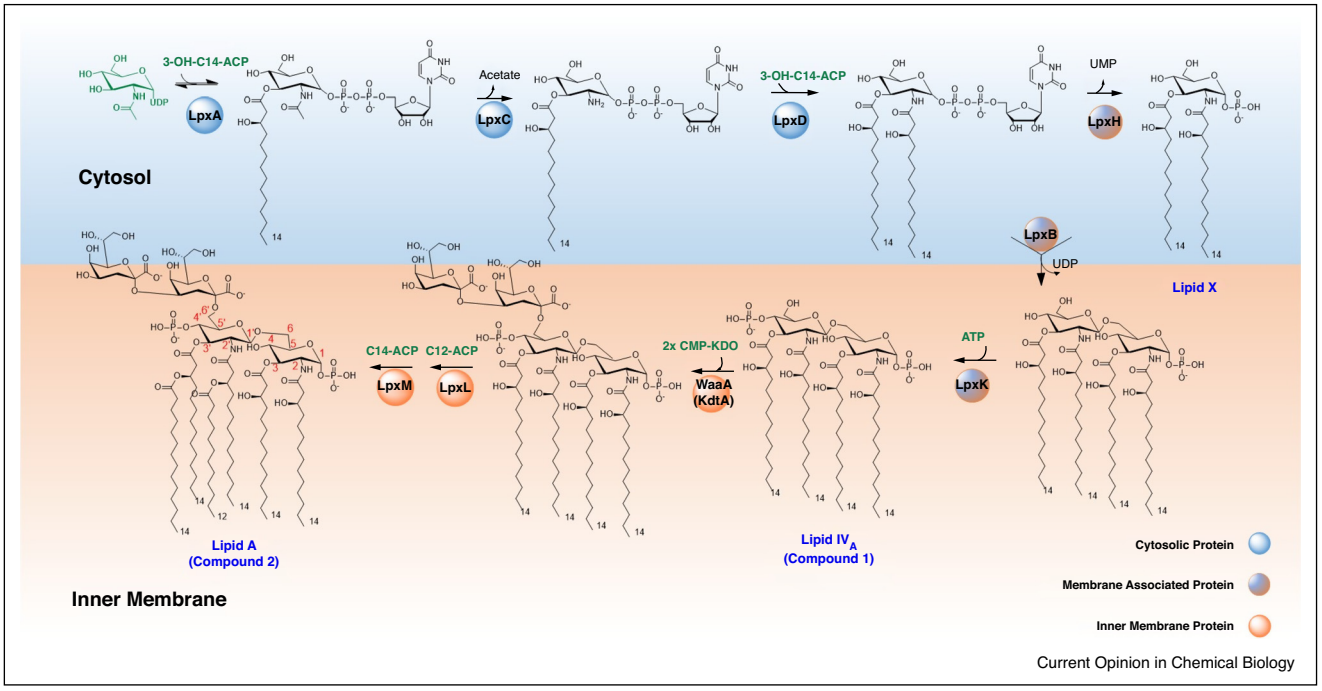
Lipopolysaccharide (LPS) is a complex glycolipid found in the outer membrane of Gram-negative bacteria. It acts as a barrier to entry of foreign molecules into the bacterium, and plays a role in maintaining the integrity of cell membrane. Lipid A, the conserved, lipid component of LPS, anchors LPS to the outer membrane. Canonical Lipid A, produced in *Escherichia coli*, is a β-(1′,6)-linked disaccharide of glucosamine that is hexa-acylated and bis-phosphorylated (Compound 2, Figure 4). The low $pK_{a,1}$ value of its phosphate groups results in the compound having at least two negative charges at neutral pH, and contribute towards a net negative surface charge [1]. As a pathogen associated molecular pattern (PAMP), Lipid A induces a range of human innate immune responses upon binding to its receptors, namely Toll-like receptor (TLR4), caspase-4, and caspase-5. These responses result in the recruitment of immune cells and fluids to the site of infection to eliminate the foreign pathogen. In some situations, Lipid A can trigger systemic inflammation that causes tissue damage and occasionally death. To evade this immune response, some Gram-negative bacteria, such as *Yersinia Pestis*, have diversified their Lipid A structures to result in attenuated inflammation. In recent years, the structural diversity of Lipid A has been harnessed to develop a vaccine adjuvant (MPL®) that safely enhances a beneficial adaptive immune response against the co-inoculated antigen [2]. This review discusses the biosynthetic diversity of Lipid A, and the sensing of Lipid A analogs by components of the host defense systems. The consolidated information provides a landscape of our current understanding of Lipid A immune response and insights into the potential therapeutic use of this class of molecules.

Biosynthesis of Lipid A in *E. coli* [3,4•]

In *E. coli*, the biosynthesis of Lipid A is catalyzed by nine enzymes (LpxA, LpxC, LpxD, LpxH, LpxB, LpxK, KdtA, LpxL, LpxM), and requires three distinct protein-bound acyl donor substrates along with UDP-*N*-acetylglucosamine (UDP-GlcNAc), ATP, and CMP-3-deoxy-D-*manno*-octulosonic acid (CMP-Kdo). The natural acyl donor substrates are fatty acyl chains attached to the acyl carrier protein (acyl-ACPs), intermediates in fatty acid biosynthesis. Hence, the Lipid A biosynthetic pathway is considered to operate downstream of fatty acid biosynthesis (Figure 1). To facilitate the trafficking of LPS to the outer membrane, Lipid A biosynthetic enzymes (LpxA through LpxM) are either expressed in the cytoplasm or anchored to the inner membrane. After core oligosaccharide-Lipid A biosynthesis, the compound is enzymatically flipped and oriented towards the periplasm for the addition of *O*-antigenic polysaccharide, and it is subsequently transported to the outer membrane (reviewed in [3]).

Figure 1



Biosynthesis of Lipid A in *E. coli* (adapted from [4•]). The enzymes LpxA, LpxC, and LpxD are located in the cytoplasm, whereas LpxH, LpxB, LpxK, WaaA, LpxL and LpxM are anchored to the inner membrane. Substrates for the biosynthetic pathway are highlighted in green: acyl carrier protein (ACP)-bound acyl donors, UDP-N-acetylglucosamine (UDP-GlcNAc), ATP, and CMP-3-deoxy-D-manno-octulosonic acid (CMP-Kdo). The product of the pathway is further modified into variable lipopolysaccharide structures via further glycosylation of the Kdo sugars (not explicitly shown).

To initiate the biosynthetic pathway, cytosolic proteins LpxA, LpxC and LpxD catalyze a series of reactions converting UDP-GlcNAc into UDP-2,3-diacyl-glucosamine. First, LpxA transfers the acyl chain from (*R*)-β-hydroxymyristoyl-ACP to the 3-OH group of UDP-GlcNAc. This acyl transfer reaction is thermodynamically unfavorable. To drive the reaction forward, LpxC, a Zn^{2+}-dependent metalloenzyme, hydrolyzes the 2-acetamido functionality, and thus catalyzes the first committed step in the pathway. LpxD then installs another (*R*)-β-hydroxymyristoyl moiety onto the 2-amino group, yielding UDP-2,3-diacyl-glucosamine.

The UDP-2,3-diacyl-glucosamine product of LpxA-C-D is transformed by three membrane associated enzymes, LpxH, LpxB and LpxK, into tetraacylated Lipid IV_A (Compound 1, Figure 4). LpxH hydrolyzes UDP-2,3-diacylglucosamine to yield 2,3-diacylglucosamine 1-phosphate. LpxB catalyzes formation of a β-1′,6-glycosidic bond to generate the disaccharide backbone. In the final step leading up to Lipid IV_A, LpxK phosphorylates the 4′-position of this disaccharide.

Prior to late-stage acylation, *E. coli* requires the addition of two 2-keto-3-deoxyoctonate (Kdo) sugars to Lipid IV_A. A bifunctional enzyme KdtA catalyzes two successive glycosyl transfer reactions to form Kdo_2-Lipid IV_A. This tetraacyl tetrasaccharide is further modified by late acyl transferases LpxL and LpxM through the addition of lauroyl and myristoyl secondary acyl chains, respectively, to yield hexaacylated Lipid A.

Biosynthetic diversity of Lipid A [3,4•]

Gram-negative bacteria have evolved an immense capacity for diversifying Lipid A structure (reviewed in [4•]). The modification system typically involves enzymes within the biosynthetic pathway and/or downstream tailoring enzymes that recognize and modify Lipid A.

Within the Lipid A biosynthetic pathway, acyl transferase orthologs have variable substrate specificities, and thus contribute towards alterations in product structure (Table 1). These enzymes recognize fatty acyl chains of different lengths and oxidation patterns. For example, *E. coli* LpxA has 100–1000 times higher specificity for (*R*)-β-hydroxymyristoyl-ACP (C14:0) than C_{12} or C_{10} acyl-ACP substrates. While *E. coli* acyl transferases are chain length specific, orthologs from other Gram-negative bacteria are more tolerant, yielding a heterogeneous Lipid A composition of the outer membrane. These enzymes also show diversity with respect to the identity of their acyl acceptors. For example, *E. coli* LpxA transfers acyl chain to UDP-GlcNAc while *Leptospira interrogans* LpxA