Lipoteichoic Acid Synthesis and Function in Gram-Positive Bacteria

Matthew G. Percy and Angelika Gründling

Section of Microbiology and MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, SW7 2AZ UK; email: m.percy09@imperial.ac.uk, a.grundling@imperial.ac.uk

Keywords
bacterial cell wall, D-alanylation, glycosylation, cholination, lipid turnover, osmoregulated periplasmic glucans

Abstract
Lipoteichoic acid (LTA) is an important cell wall polymer found in gram-positive bacteria. Although the exact role of LTA is unknown, mutants display significant growth and physiological defects. Additionally, modification of the LTA backbone structure can provide protection against cationic antimicrobial peptides. This review provides an overview of the different LTA types and their chemical structures and synthesis pathways. The occurrence and mechanisms of LTA modifications with D-alanyl, glycosyl, and phosphocholine residues will be discussed along with their functions. Similarities between the production of type I LTA and osmoregulated periplasmic glucans in gram-negative bacteria are highlighted, indicating that LTA should perhaps be compared to these polymers rather than lipopolysaccharide, as is presently the case. Lastly, current efforts to use LTAs as vaccine candidates, synthesis proteins as novel antimicrobial targets, and LTA mutant strains as improved probiotics are highlighted.
INTRODUCTION

Teichoic acids (TAs) were first detected by Baddiley and coworkers in 1958 (6, 7). A structural diversity was soon recognized, and the term TA, derived from the Greek word *teichos*, for “wall,” was subsequently used to describe all bacterial cell wall, membrane, and capsular polymers containing glycerolphosphate (GroP) or ribitolphosphate (RboP) residues (8). Currently the term TA describes two bacterial cell wall polymers found in gram-positive bacteria: wall teichoic acid (WTA) and lipoteichoic acid (LTA). WTA synthesis has recently been reviewed (17), and here we focus only on LTA synthesis. LTA is now defined as an alditolphosphate-containing polymer that is linked via a lipid anchor to the membrane in gram-positive bacteria. However, this definition may need to be revised to include recently described complex glycosyl-phosphate-containing polymers (107). LTAs have been grouped into different types based on their chemical structures (Figure 1). This review focuses on the synthesis, modifications, and functions of type I and type IV LTAs, as these have been studied in the greatest detail. Furthermore, we summarize recent attempts to utilize LTAs as vaccine candidates, LTA mutant strains as improved probiotic strains, and biosynthetic enzymes as antimicrobial targets. Lastly, we highlight similarities between the synthesis pathways of LTA and osmoregulated periplasmic glucans (OPGs) of gram-negative bacteria.

LTA STRUCTURES AND THEIR SYNTHESIS AND FUNCTION

Polyglycerolphosphate (Type I) LTA

Polyglycerolphosphate, or type I LTA, is the best-characterized LTA. It is found in a large range of gram-positive bacteria belonging to the phylum *Firmicutes*, such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Type I LTA has an unbranched 1–3 linked GroP backbone
a Type I LTA

b Type II LTA

c Type III LTA

d Type IV LTA

e Type V LTA
Figure 2

Type I LTA synthesis machinery and lipid turnover in Staphylococcus aureus. The glycolipid anchor Glc2-DAG is produced by YpfP and moved to the outside of the membrane, likely by LtaA. The LtaS enzyme produces the polyglycerolphosphate chain by the repeated addition of GroP residues to the tip of the growing chain using the lipid phosphatidylglycerol (PG) as substrate. The concomitantly formed DAG is recycled in the cytoplasm to PG in reactions catalyzed by DgkB, CdsA, PgsA, and as of yet unknown enzyme(s) with phosphatidylglycerol phosphate phosphatase activity. The scissors indicate the processing step of LtaS by the type I signal peptidase SpsB. This figure was adapted with permission from Reichmann & Gründling (106). The publisher for this copyrighted material is John Wiley and Sons. Abbreviations: DAG, diacylglycerol; Glc2, diglucosyl; GroP, glycerolphosphate; LTA, lipoteichoic acid.
enzymes; however, the two-enzyme system found in *Listeria* spp. appears to be unique, as LtaP has significantly diverged from the LtaS enzyme. In *Bacillus* spp., which contain four LTA synthase enzymes, these proteins are more closely related to each other and the LtaS protein of *L. monocytogenes* than to LtaP.

Disaccharide-containing glycolipids are often the predominant glycolipids and are present both as free membrane lipids and as LTA anchors such as β-glycosyl(1–6)-β-glucosyl(1–3)-diacylglycerol (Glc₂-DAG) in *S. aureus* and *B. subtilis* or α-galactosyl(1–2)-α-glucosyl(1–3)-DAG in *L. monocytogenes* (34, 59, 60, 66, 69, 123, 128). However, glycolipids with mono-, tri-, or tetrasaccharides are also found in bacterial membranes and are used as LTA anchors, and they can be further substituted with acyl chains or fatty acids (33). A growth temperature–dependent variation in the LTA lipid anchor has recently been observed for *L. monocytogenes* (28). The anchor contained a second DAG lipid when the bacteria were grown at 37°C, and this LTA polymer showed reduced binding to the eukaryotic pattern-recognition protein L-ficolin (28). A comprehensive review covering the diversity of glycolipids and phosphoglycolipids and their interrelationship with LTA synthesis was published some years ago but is still relevant (33). Enzymes involved in glycolipid synthesis were covered in a recent review and are not further discussed here (106).

Mutations in *ltaS* have now been generated in *S. aureus*, *B. subtilis*, *Bacillus anthracis*, *L. monocytogenes*, and *Lactobacillus acidophilus* (39, 44, 91, 97, 110, 128, 131). With the exception of *L. acidophilus*, strains either lacking LTA or with defects in LTA backbone synthesis have severe growth defects. *L. monocytogenes*, *B. subtilis*, and *B. anthracis ltaS* mutants display a filamentation phenotype, which in *B. subtilis* was attributed to the inability of the key cell division protein FtsZ to assemble at the division site (39, 110, 128, 131). In addition, evidence of a direct interaction between cell division proteins and LTA synthesis proteins was recently provided for *S. aureus* (105). An *S. aureus ltaS* mutant can only be maintained under osmotically stabilizing conditions in medium with a high concentration of NaCl or sucrose, after acquisition of compensatory mutations, or, for some strains, by lowering the growth temperature (22, 44, 97). The observation that an *S. aureus ltaS* mutant can be rescued in high-osmolality medium or by an increase in the cellular concentration of cyclic-diadenosine-monophosphate (c-di-AMP), a nucleotide-signaling molecule that has been implicated in the control of potassium and/or other ion transport (9, 22–24, 94, 97), might indicate a crucial function of LTA in the osmoprotection of the cell.

**Type I LTA Synthesis and Lipid Turnover**

Type I LTA synthesis is intimately linked with lipid biosynthesis and turnover, as has been described in detail for *S. aureus* (69). In this organism, every ninth lipid on the outer leaflet of the membrane is an LTA molecule with an average length of 25 GroP units (69). It has been estimated that the PG pool is turned over more than twice per generation time to support LTA synthesis (69). For each PG molecule that is hydrolyzed to extend the LTA chain by one repeating unit, one molecule of DAG is produced. DAG then reenters the cell, where it is recycled to PG or used for the synthesis of glycolipids (Figure 2). In the first step of the recycling process, the cytoplasmic enzyme DgkB converts DAG to phosphatidic acid (57, 58, 90) (Figure 2). Phosphatidic acid is subsequently converted to PG via the conventional PG synthesis pathway by the successive actions of the phosphatidate cytidyltransferase CdsA, the phosphatidylglycerol phosphate synthase PgsA, and one or more enzymes with phosphatidylglycerol phosphate phosphatase activity that convert phosphatidylglycerol phosphate to PG (Figure 2). Although three enzymes, PgpA, PgpB, and PgpC (52, 53, 78), with this activity have been characterized in *E. coli*, the enzyme(s) with such activity has not yet been identified in gram-positive bacteria.
DgkB activity is essential for the growth of *B. subtilis*; in a conditional dgkB mutant strain, LTA synthesis ceased and DAG accumulated rapidly (57). However, the lethality was prevented by the disruption of either of the two main LTA synthase enzymes, YfE or YfnI (87). Thus, DgkB activity and the DAG recycling pathway are only essential in cells with a high PG turnover caused by LTA synthesis, potentially indicating that the accumulation of DAG is not tolerated by the cell. However, inactivation of YfnI does not result in a decrease in DAG levels, and therefore the accumulation of DAG alone cannot explain the growth arrest seen in the dgkB mutant (87).

**Complex LTA Structures**

Several gram-positive bacteria produce more complex LTA polymers, here referred to as type II–V LTAs (Figure 1). Significant progress has been made toward understanding the structure, synthesis, and function of type IV LTA found in *Streptococcus pneumoniae* (see the following section). As with type IV LTA, the chemical structures of type II, III, and V LTAs may undergo further revisions once they are subjected to additional analysis. It is also likely that additional complex LTAs will be discovered. Type II LTA, found in *Lactococcus garvieae* strain Kiel 4217, has a proposed backbone of α-Gal(1–6)-α-Gal(1–3)-GroP repeating units in which the GroP units can be further substituted with α-Gal residues (68). The polymer is linked via an α-Glc(1–2)-α-Glc(1–3)-DAG anchor to the membrane, and the first glucose (Glc) molecule can carry an additional acyl chain (68) (Figure 1). Type III LTA, found in *Clostridium innocuum*, has a proposed structure of α-Gal(1–3)-GroP–repeating units linked via a β-glucosamine(1–3)-α-Glc(1–3)-DAG lipid anchor to the membrane. The GroP residues are substituted with either N-acetylglucosamine (25%) or glucosamine (50%) residues (35). An LTA polymer with yet another structure, type V LTA, has been observed in *Peptostreptococcus anaerobius* and *C. difficile* (107, 114). Type V LTA has a proposed structure of α-D-GlcNAc(1–3)-α-D-GlcNAc repeating units linked through C-6′-C-6″ phosphodiester bridges. The second N-acetylglucosamine (GlcNAc) residue is further decorated with D-glyceric acid, and the polymer is retained in the membrane via a β-1-6-linked triglycosyl-DAG glycolipid anchor (107) (Figure 1). The enzymes involved in type II, III, and V LTA synthesis are unknown. When and if the structures are confirmed, the definition of LTA as alditolphosphate-containing polymers might need to be revised to include glycosyl-phosphate-containing polymers such as type V LTA.

**Streptococcus Pneumoniae (Type IV) LTA**

Type IV LTA is found in *S. pneumoniae* and some other *Streptococcus* spp. Pneumococcal LTA (pnLTA) was described in 1930 as pneumococcal C-polysaccharide (119) and was later shown to possess Forssman antigenicity (41). This observation was only explained in 2008, when a revised structure with two terminal GlcNAc residues was proposed; these represent the minimal Forssman antigen (111). In 2013, additional revisions were proposed (40), and it is now assumed that the backbone consists of pseudopentasaccharide repeating units made up of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AATGal), Glc, RboP, and two GlcNAc residues (Figure 1). The repeating units are α-1-4-linked to each other and β-1-4-linked to the glycolipid anchor α-Glc(1–3)-DAG (40, 111). It was further suggested that pnLTA contains only d-alanine (D-Ala) modifications on the RboP residues and is not glycosylated, as originally proposed (40, 71).

Most bacteria containing LTA also synthesize WTA. On the one hand, in organisms with type I LTA, the two polymers have different structures and are synthesized by different enzymes. pnLTA and pnWTA, on the other hand, have identical structures and are produced by the same enzyme machinery. A detailed model for their synthesis has been proposed (Figure 3) (29); however, many
Type IV LTA synthesis machinery in *Streptococcus pneumoniae*. The enzymes Spr1655, Spr0091, LicD3 and Spr1123, and Spr1124 use the indicated nucleotide-activated sugars or ribitolphosphate (RboP) as substrate for the synthesis of the C55-P-linked pseudopentasaccharide intermediate. The N-acetylglucosamine (GlcNAc) residues are decorated with phosphocholine (P-Cho) residues by LicD1 and LicD2. The chain is polymerized by Spr1222, transported across the membrane by TacF, and transferred onto either the glycolipid Glc-DAG (LTA) or peptidoglycan (WTA) by LCP family enzymes. This figure was adapted with permission from Denapaite et al. (29) The publisher for this copyrighted material is Mary Ann Liebert, Inc., publishers. Abbreviations: Glc-DAG, glucosyl-diacylglycerol; LCP, LytR-CpsA-Psr; LTA, lipoteichoic acid.

MODIFICATIONS OF LTAS AND THEIR FUNCTIONS

**D-Alanylation of LTA**

Type I and IV LTAs (and WTAs) are modified with D-Ala residues, and the enzymes required for this process are encoded by the *dltABCD* operon (46, 95). The D-alanylation process starts in the cytoplasm of the cell, where the d-alanine-d-alanyl carrier protein ligase DltA activates D-Ala using ATP to form a high-energy d-alanyl-AMP intermediate and subsequently transfers it to the phosphopantheinyll prosthetic group of the d-Ala carrier protein DltC (Figure 4a) (30, 98, 134). DltC shows sequence and structural homology to acyl carrier proteins (ACP) (124), and structural studies on DltA have shown that this protein belongs to the adenylate-forming

**Figure 3**

Changes may still occur before final publication online and in print
enzyme family (30, 98, 134). DltA is assumed to undergo major structural rearrangements during the reaction cycle that are driven by small changes in electrostatic interactions introduced by substrate binding and product formation. The function of the remaining proteins, DltB and DltD, is not completely understood, and different models have been proposed (27, 95, 100). Recent work has provided experimental support for a model originally proposed by Werner Fischer and colleagues (34, 100, 104). In this model, the multiple-membrane-spanning protein DltB transfers
the D-Ala from DltC to C55-P and subsequently transports the D-Ala-P-C55 intermediate across the membrane (Figure 4a). DltB has been grouped among membrane-bound O-acyltransferase (MBOAT) proteins, where other members are known to transfer organic acids onto hydroxyl groups of membrane-embedded components or reacylate lysophospholipids (50, 112). DltD has, as recently shown, an N-in C-out membrane topology placing the functional part of the protein on the outside of the cell (104). In a previous study, thioesterase activity was ascribed to DltD on a cytoplasmic substrate and was unrelated to this pathway (27). The new findings and the location of DltD on the outside of the cell, however, might suggest that this protein is the enzyme that aids in the final step and transfers the D-Ala onto LTA (Figure 4a).

A number of environmental factors have been shown to influence the amount of D-alanines on LTA. An increase in pH (79), temperature (51), or NaCl (37) leads to a decrease in D-alanylation. At high pH the ester linkage is labile. The half-life of D-alanines on LTA is 3.9 h at pH 8, whereas it is >10,000 h at pH 6 (20). Two-component systems play a crucial role in regulating the expression of the dlt operon. In S. aureus, the expression of the dlt operon is repressed by ArlSR, in response to an increase in the concentration of Mg²⁺ (70). The response is, however, species specific: An increase in Mg²⁺ or K⁺ has no effect on dlt gene expression in Streptococcus gondii (89). The LiaSR two-component system, which responds to cell membrane damage (115), induces the expression of the dlt operon in response to polymyxin B as well as low pH in S. gondii (89), and in Streptococcus agalactiae, dlt expression is controlled by the DltRS system (103).

Analysis of dlt mutants in diverse bacteria revealed that this modification plays an important role in the regulation of autolysis (99, 113), cation homeostasis through the binding of Mg²⁺ ions (5), host cell adhesion and invasion (1, 72), and biofilm formation (32) and is essential for the virulence of pathogens such as L. monocytogenes and S. aureus (1, 21). In addition, D-alanylation of TAs provides resistance to cationic antimicrobial peptides (CAMPs) (1, 102). It has been presumed that the presence of D-alanines raises the overall net negative charge of the membrane, thereby reducing the affinity for CAMPs. However a recent study on Streptococcus pyogenes questioned the link between charge and the ability of CAMPs to cross the membrane, suggesting instead that the D-alanines alter the conformation of LTA, leading to an increase in the density of the cell wall (109) (see also sidebar, Amino Acid Modifications of Lipopolysaccharides in Gram-Negative Bacteria).

**Glycosylation of LTA**

A mechanism for the incorporation of glycosyl residues into type I LTA has been proposed based on work performed in the 1980s (34, 55, 81, 133). Some but not all type I LTAs are glycosylated, and variations are also observed in the type of sugar used for this modification. This has been

---

**Figure 4**

LTA modifications. (a) D-Ala modification mechanism of type I and IV LTAs based on the Fischer model (100). DltA ligates D-alanines onto DltC. The D-alanines are transferred onto undecaprenyl phosphate (C55-P) and subsequently transported across the membrane by DltB. DltD then aids in the transfer of the D-alanines to glycerolphosphate (GroP) of type I or ribitolphosphate (RboP) of type IV LTA. (b) Proposed glycosylation mechanism of type I LTA. A cytoplasmic glycosyltransferase (likely a GT-A member) uses a nucleotide-activated sugar such as UDP-GlcNAc and links it onto C55-P. The intermediate is transported across the membrane and an extracellular glycosyltransferase (likely a GT-C member) then transfers the sugar onto LTA. (c) Phosphocholine (P-Cho) modification mechanism of type IV LTA. Choline is transported into the cell by LicB and converted to P-Cho and CDP-Cho by the action of LicA and LicC. The enzymes LicD1 and LicD2 then use CDP-Cho to substitute the N-acetylglucosamine (GlcNAc) residues of the TA precursors with P-Cho residues. Abbreviation: LTA, lipoteichoic acid.
AMINO ACID MODIFICATIONS OF LIPOPOLYSACCHARIDES IN GRAM-NEGATIVE BACTERIA

Proteins with homology to DltA–D have been detected in some gram-negative bacteria belonging to the genera *Erwinia*, *Photorhabdus*, and *Bordetella* (2). The genes were likely acquired by horizontal gene transfer, as their CG content differs from that of the rest of the genome. A *Bordetella pertussis* strain with a deletion of this D-alanine incorporation and resistance to AMPs, or dra, operon has been produced (2). Reminiscent of phenotypes observed for dlt mutants in gram-positive bacteria, the dra mutant showed increased susceptibility to antimicrobial peptides and was killed more readily by human phagocytes. Initial experiments suggest that outer membrane proteins might be the targets of this modification (2). Another amino acid modification has been observed on the lipid A anchor of lipopolysaccharide in *Vibrio cholerae* and compared to the Dlt system in gram-positive bacteria (45). Three proteins, AlmE–G, are required for the introduction of these glycine and diglycine modifications. AlmE shows sequence homology to DltA, and it activates and transfers the glycines onto the small carrier protein AlmF (45). AlmF does not show sequence homology to DltC but has been suggested to possess a similar fold. The glycine residues are then transferred from AlmF onto the lipid A anchor by AlmG (45).

**Phosphocholeline Modification of Type IV LTA**

Type IV LTA in *S. pneumoniae* is modified with P-Cho residues (29, 120). In contrast to D-alanylation, this modification is essential for bacterial growth. The P-Cho residues are introduced
into the TA precursors within the cytoplasm of the cell, and it is assumed that the polymer is only exported once it is modified with P-Cho (26). Choline is transported into the cell by LicB and converted into P-Cho and subsequently CDP-Cho by LicA and LicC, respectively, and then attached to the O-6 position of one or both GalNAc residues by the enzymes LicD1 and LicD2 (Figure 4c) (10, 31, 36, 135). Once the modified polymer is exported, the P-Cho content can be reduced by the phosphorylcholine esterase Pce (47, 125). Because of the structure of its active site, this enzyme is only capable of removing the terminal choline residues, and it has been suggested that the reduction in choline residues exposed on the bacterial surface is important to prevent the interaction with host immune proteins (47).

The growth requirement of \(S.\ pneumoniae\) for choline can be counteracted by the addition of other amino-alcohols such as ethanolamine, although cells grown under these conditions have various morphological defects and are attenuated in virulence (121). The amino-alcohol physically replaces the P-Cho residues on the TAs, but it cannot replace them functionally. Several cell wall proteins, collectively referred to as choline-binding proteins (CBPs), require P-Cho for their retention in the cell wall or even activity, as in the case of LytA (38, 122). CBPs are modular enzymes that in several cases contain a peptidoglycan hydrolase domain and a variable number of choline-binding domains (38). These enzymes are required for the remodeling of the peptidoglycan, autolysis, and natural competence (122). Structural analyses of CBPs have provided insight into the binding and substrate specificity of this class of enzymes (47, 92, 101). CBPs also play a significant role in the virulence of \(S.\ pneumoniae\), as some CBPs, including PspA, PspC, and CbpG, mediate interactions with host cells (64, 82, 96, 126). PspA has an additional function: It also prevents complement deposition on the pneumococcal surface by inhibiting the binding of the C-reactive host immune protein (CRP) to bacteria by competing for P-Cho-binding sites (93). Taken together, these findings make it clear that the P-Cho modifications on TAs play a key role in bacterial physiologi and the virulence of \(S.\ pneumoniae\); however, it should be noted that one cannot distinguish between the requirement of this modification on LTA versus WTA.

APPLICATIONS OF LTA AND MUTANT STRAINS

Several lines of research that are currently being pursued could be of direct clinical relevance. LTA polymers and specific antibodies against these polymers have been tested as vaccine candidates. Immunization of mice with gram-positive bacteria or purified LTA has been shown to elicit the production of opsonic antibodies (67, 117). To increase the immunogenicity of LTAs, the production of conjugate vaccines is currently being pursued. Recent studies have reported on the synthesis of a conjugate between synthetic type I LTA and the immunogenic tetanus toxin protein or between type V LTA of \(C.\ difficile\) with \(E.\ coli\) enterotoxin or a \(Pseudomonas\) exotoxin (13, 18, 25). Much effort has gone into the use of type I LTA-specific antibodies in passive immunization studies (118, 129, 130). A humanized antibody has been used in human trials in very-low-weight neonates, and the vaccine appeared safe and well tolerated; however, no clear protection was observed against staphylococcal sepsis in this study (129, 130). Enzymes involved in LTA synthesis and their modifications can also be exploited as antimicrobial targets. For instance, choline analogues have been shown to bind to and inhibit the activity of pneumococcal CBPs (80, 83). A d-Ala analog that blocks the activity of DltA in vitro has been shown to synergize with the peptidoglycan synthesis inhibitor vancomycin and lead to growth inhibition when used in combination (88). Recently, an LtaS enzyme inhibitor was identified (108). This small molecule was shown to prevent the growth of \(S.\ aureus\) and several other antibiotic-resistant gram-positive bacteria (108). Mice challenged with a lethal dose of \(S.\ aureus\) showed increased survival in the presence of this compound (108). The identification of the genetic determinants required for
LTA production made it possible to compare the interaction of wild-type and LTA-deficient bacteria with immune cells. Recent work using an LTA-negative L. acidophilus strain highlighted the potential use of such a mutant as a probiotic strain with improved properties (75). In contrast to wild-type L. acidophilus, the ltaS mutant strain was shown to downregulate the production of the inflammatory cytokines IL-12 and TNFα and enhance the production of the anti-inflammatory cytokine IL-10 (91). Colonization of mice with the LTA-deficient L. acidophilus strain also showed promising results: This treatment mitigated the effects of induced colitis, highlighting the potential use of such a strain for the treatment of inflammatory intestinal disorders (91). More recently, its application in preventing colon cancer is also being pursued (65).

**COMPARISON OF TYPE I LTA AND OSMOREGULATED PERIPLASMIC GLUCAN SYNTHESIS**

LTA is often compared to LPS of gram-negative bacteria. In particular for type I LTA, it might be more appropriate to compare this polymer to OPGs of gram-negative bacteria, based on similarities between enzymes involved in their synthesis, their cellular location within the periplasm (they are not surface-exposed molecules (105)), and their function in osmoprotection. OPGs, formerly referred to as membrane-derived oligosaccharides (MDOs), are oligosaccharides that accumulate under low osmolarity conditions in the periplasm of gram-negative bacteria (63). In E. coli, linear β(1–2)-linked glucose units are produced as lipid-linked intermediates by the glycosyltransferase OpgH. Similar to the LTA glycolipid anchor–producing enzyme YpfP of S. aureus (or UgtP of B. subtilis), this enzyme uses UDP-glucose as a substrate. OpgH has also been shown to play a key role in nutrient-dependent and UDP-glucose-concentration-dependent cell size control in E. coli (49). A similar function has been previously reported for the B. subtilis UgtP enzyme through regulating the polymerization of the cell division protein FtsZ (19, 127). The linear lipid-linked glucose chains are then transported to the outer leaflet of the inner membrane where the periplasmically located glycosyltransferase OpgG adds β(1–6)-linked glucose branches. The oligosaccharides are then decorated with succinyl, phosphoethanolamine, and GroP residues by the action of OpgC, OpgE, and OpgB, respectively (15, 63). OpgB, also referred to as MdoB, shares many features with LtaS-type enzymes. Both enzymes consist of an N-terminal transmembrane and a C-terminal extracellular enzymatic domain (74). Similar to LtaS, OpgB is processed during bacterial growth by a protease speculated to be the signal peptidase, and the enzymatic domain is released into the periplasmic space (74). Both enzymes are Mn2+–dependent metal enzymes that use the lipid PG as substrate to add the GroP units onto the periplasmic oligosaccharides or onto the glycolipid anchor, respectively (42, 56). In both instances, this leads to a rapid PG lipid turnover and the requirement of a functional lipid-recycling pathway. Based on cryoelectron microscopy images, LTA has been suggested to be an important constituent of the inner cell wall zone also referred to as the gram-positive periplasm (84–86). OPGs accumulate in the gram-negative periplasm in low-osmolarity medium and are thought to protect bacteria under these conditions, although only modest growth defects have been observed in their absence (14, 63). The observation that an S. aureus strain lacking LTA can be rescued in high-osmolality medium or by an increase in the cellular c-di-AMP concentration, implicated in the control of ion transport (22, 23, 97), might indicate a similar function for LTA in the osmoprotection of the cell.

**CONCLUSIONS**

LTA represents a significant proportion of the cell membrane and inner cell wall zone in gram-positive bacteria. It plays an important role in bacterial growth and physiology and contributes to
Although we now have a good understanding of type I and IV LTA synthesis and their modifications with d-Ala and P-Cho residues, more work is needed on the glycosylation mechanism and the synthesis pathways of other complex LTAs. Recent advances in the field have allowed the investigation of these polymers as vaccine candidates, LTA mutant strains as probiotics with improved characteristics, and LTA synthesis enzymes as novel antimicrobial targets.

**SUMMARY POINTS**

1. LTA is defined as an alditolphosphate-containing polymer that is linked via a lipid anchor to the outside of the membrane in gram-positive bacteria. However, this definition may need to be revised to include recently described complex glycosyl-phosphate containing polymers.

2. LTAs have been grouped into different types based on their chemical structures. Type I LTA has a simple unbranched polyglycerolphosphate backbone structure whereas type II–V LTAs have more complex structures.

3. Type I polyglycerolphosphate LTA production is intimately linked with membrane lipid turnover, and its synthesis shows striking similarities to the production of osmoregulated periplasmic glucans in gram-negative bacteria.

4. LTA plays an important role for bacterial growth and physiology and contributes to membrane homeostasis and virulence.

5. The essential nature of LTA makes it an attractive target for vaccines and novel antimicrobials.

**FUTURE ISSUES**

1. Based on bioinformatics analysis, several enzymes have been predicted to be involved in type IV LTA synthesis; however, these need to be confirmed experimentally.

2. Further analysis is needed to confirm the chemical structures of other complex LTA types, and the enzymes required for their synthesis need to be identified.

3. DltB is thought to produce a d-Ala-P-C55 membrane intermediate, and DltD is thought to transfer d-Ala onto LTA; however, these activities need to be confirmed experimentally.

4. The genetic determinants required for the glycosylation process of LTA need to be identified and a function for this modification elucidated.

5. Further work is needed to validate LTA synthesis enzymes as a therapeutic target, LTA polymers as vaccine candidates, and LTA mutant strains as probiotics with improved characteristics.

**DISCLOSURE STATEMENT**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.
ACKNOWLEDGMENTS

Work in the A.G. laboratory is currently supported by the European Research Council grant 260371 and the Wellcome Trust grant 100289.

LITERATURE CITED


