Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides

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1. Introduction

Antimicrobial peptides (AMPs) are part of humoral immunity of the innate immune response that is an old evolutionary defense strategy of organisms to defend against attack by other organisms/pathogens. They act as antibiotics or fungicides to potentially kill bacteria and fungi, but some of them are also active against viruses and cancer cells. Their mechanism of action mostly relates to targeting the microbial

**A B S T R A C T**

A number of cationic antimicrobial peptides, effectors of innate immunity, are supposed to act at the cytoplasmic membrane leading to permeabilization and eventually membrane disruption. Thereby, interaction of antimicrobial peptides with anionic membrane phospholipids is considered to be a key factor in killing of bacteria. Recently, evidence was provided that killing takes place only when bacterial cell membranes are completely saturated with peptides. This adds to an ongoing debate, which role cell wall components such as peptidoglycan, lipoteichoic acid and lipopolysaccharide may play in the killing event, i.e. if they rather entrap or facilitate antimicrobial peptides access to the cytoplasmic membrane. Therefore, in this review we focused on the impact of Gram-positive cell wall components for the mode of action and activity of antimicrobial peptides as well as in innate immunity. This led us to conclude that interaction of antimicrobial peptides with peptidoglycan may not contribute to a reduction of their antimicrobial activity, whereas interaction with anionic lipoteichoic acids may reduce the local concentration of antimicrobial peptides on the cytoplasmic membrane necessary for sufficient destabilization of the membranes and bacterial killing. Further affinity studies of antimicrobial peptides toward the different cell wall as well as membrane components will be needed to address this problem on a quantitative level. This article is part of a Special Issue entitled: Antimicrobial peptides edited by Karl Lohner and Kai Hilpert.

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However, the mode of action of AMPs is also strongly related to cellular envelope constituents that are different and variable through diverse microbial families (Fig. 1, Table 1). In contrast to higher living organism and mycoplasma, microbial plasma membranes are surrounded by a cell wall of a tight and flexible layer composed of polysaccharides, peptidoglycan (PGN) in bacteria and glucosamine polymer chitin and β-glucan in fungi. The cell wall of Gram-positive and the outer membrane in Gram-negative bacteria contain anionic lipid molecules, lipoteichoic acid (LTA) and lipopolysaccharide (LPS) that may compete with the plasma membrane for the interaction with AMPs. Not only the cell walls, but also the plasma membrane, which matrix is formed by a phospholipid bilayer differing in headgroup and fatty acid composition contributes to mechanistic diversity of AMPs against microbial cells. Whereas bacterial plasma membranes are negatively charged due to the presence of anionic phospholipids, fungal membranes are more similar to neutral and rigid eukaryotic membranes because of their zwitterionic phospholipid constituents and ergosterol. The strong affinity to microbial membranes is also due to the transmembrane potential determined by the differences in inner and outer leaflet composition of microbial membranes and different charge density of phospholipids that promotes peptides insertion [8,12].

Although electrostatic interaction of AMPs with plasma membrane phospholipids, insertion and in turn membrane disruption is widely accepted for explaining the bacterial killing mechanism by a number of antimicrobial peptides, the pertinent question arising is to which extent antimicrobial peptides interact with microbial cell wall components that may affect the extent of their activity and functionality. Freire et al. [13] concluded that in the end the role of bacterial cell wall components as electrostatic barriers capturing AMPs and hence preventing their interaction with the cytoplasmic membrane is a matter of concentrations of AMPs and membrane components as well as affinities of AMPs toward the different membrane components. In this context, Roversi et al. [14] showed an extremely high coverage of both leaflets of the outer and inner Escherichia coli membranes by PMA-23, a cationic amphipathic helix from the cathelicidin family. Bacterial killing started at a molar ratio of bound peptide per lipid of about 1:30 and all bacteria were killed at a molar ratio of 1:4, corresponding closely to the numbers estimated by Castanho and co-workers [8] for other peptides, based on the partition constants derived from binding studies on model membranes. Therefore in this review, we will discuss the role of bacterial cell wall components interfering with antimicrobial activity either as molecules that may entrap AMPs to prevent their interaction with the inner lipid bilayer or in case of aggregation of AMPs to facilitate membrane interaction by accumulating AMPs on the surface and act via a “sponge like effect” to attract them onto the membrane interface.

2. Bacterial envelopes

Beyond the classification of bacteria according to Gram staining of PGN, Gram-positive bacteria distinguish in many features from Gram-negative bacteria [15,16] (Fig. 1, Table 1). Characteristic for both classes is that their cytoplasmic membrane is surrounded by a cell wall. Between those two compartments is the periplasmatic space or periplasm containing a wide variety of ions and proteins that are needed for numerous functions involving cellular (electron) transport, substrate hydrolysis, degradation and detoxification. In Gram-negative bacteria the periplasm occupies the space between the plasma membrane and the outer membrane. The presence of the outer membrane in Gram-negative bacteria adjacent to the periplasmatic space is the major difference between those bacterial classes as it does not exist in Gram-positive bacteria. This outer membrane is a lipid bilayer, where the inner leaflet is composed of phospholipids and the outer leaflet of lipopolysaccharides (LPS) [17–19]. In both lineages, the cell wall contains PGN layers that stabilize the cell membranes. The cell wall of Gram-positive bacteria is made of many PGN layers of about 40–80 nm that is drastically thicker than the single layered 7–8 nm thick cell wall of Gram-negative bacteria.

![Fig. 1. Cell envelopes of various microbial families.](image-url)
Peptidoglycan, a cell wall mesh

PGN because of its rigidity determines the strength and cellular shape of bacteria. Without PGN as it was shown in a production of sphero- 
plast in E. coli [22] and L-form bacteria from Bacillus subtilis [23], 
cells lose their characteristic shape. PGN as a multi-gigadalton bag-like 
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3. Peptidoglycan, a cell wall mesh

PGN because of its rigidity determines the strength and cellular shape of bacteria. Without PGN as it was shown in a production of spheroplast in E. coli [22] and L-form bacteria from Bacillus subtilis [23], cells lose their characteristic shape. PGN as a multi-gigadalton bag-like molecule accounts for around 90% of dry weight in Gram-positive and 10% in Gram-negative bacteria. The molecular weight of single layered E. coli PGN sacculus is \(3 \times 10^3\) Da, which is in the same range as a chromosome (2.32 \( \times 10^9\) Da) of this bacteria [20]. In Gram-positive bacteria PGNs make up to 40–80 layers. PGN is composed of alternating units of disaccharide N-acetyl glucosamine – N-acetyl muramic acid (NAM – NAG) cross-linked by a pentapeptide side chain (stem) [22] (Fig. 3). The pentapeptide has usually the sequence L-alanyl-γ-D-glutamyl 
diaminopimelil (or L-lysyl)-D-alanyl-D-alanine. In Gram-positive 
bacteria an inter-bridge structure of five amino acid residues that varies 
between the species (e.g. five glycine molecules in Staphylococcus aureus) links two disaccharide-pentapeptide moieties [24]. PGN synthesis 
starts on the cytosolic side of bacterial cell membrane from the common 
building block, lipid II (for graphic illustrations see review [25]) that 
consist of a polyisoprenoid anchor of C55 carbon chain (11 subunit long) 
attached to one disaccharide–pentapeptide subunit via pyro磷酸ate linkage. Lipid II monomer is translocated to the periplasmic 
(exterior) side of the bacterial cell membrane for incorporation into 
the growing PGN network. Different findings and models for organization 
of the PGN murein sacculus have been proposed and it has been a 
matter of debate, if murein glycans and peptides are arranged parallel 
(layered model) or perpendicular (scaffold model) to the membrane 
[20,26,27]. Recent NMR studies revealed that the disaccharide backbone 
of Gram-positive bacteria adopts 4-fold screw helical symmetry with 
disaccharide unit periodicity of 4 nm, where each PGN stem is oriented 
90° in respect to the previous stem [24]. The lattice of cross-linked stems 
has parallel orientation.

3.1. Peptidoglycan, a cell wall “sponge” attracting antimicrobial peptides in Gram-positive bacteria

The role of PGN in respect of interaction with antimicrobial peptides is 
not well understood. The literature often reports studies performed with 
proteins, which also exhibit antimicrobial activity and use PGN as a target 
for pathogen recognition. This was reported for proteins as lectins and 
natural or semi-synthetic antibiotics like glycopeptides bearing unusual 
aminos acids or modifications [28,29]. They bind to multiple sites in the 
PGN and in turn interfere with further enzymatic processes resulting in 
inhibition of PGN synthesis. Examples also include the branched tricyclic 
glycopeptide vancomycin [30] and lipoglycodepsipeptides like the mac-
rocyclic ramoplanin derived from Actinoplanes sp. [29]. Vancomycin 
was developed in the 1950s and was viewed by many as a gold standard 
for treatment of methicillin resistant S. aureus (MRSA) infections and its 
analogues [30]. The mode of action on glycolipids can be exemplified by oritavancin, a semisynthetic lipoglycopeptide analogue of vancomycin, which displays a set of sequential mechanisms ranging from inhibition of PGN synthesis, perturbation of the membrane integrity to bacterial activity against Gram-positive organisms. Oritavancin binds to the alanine—
alanine stem of the pentapeptide moiety of lipid II and also to the pentaglycyl bridging segment that inhibits PGN synthesis via inhibition of transglycosylation and transpeptidation [31]. In contrast to its analogue vancomycin, the 4′-chlorobiphenyl group of oritavancin allows interaction with lipid II and cell membrane anchoring, which results in perturbation of the cell membrane integrity in *S. aureus* and *Enterococcus faecalis* [32]. Using the fluorescence indicator 3,3′-dipropylthiacarbocyanine [32,33], membrane depolarization in *S. aureus* following the exposure to oritavancin was measured showing that oritavancin is able to depolarize the plasma membrane. In a “live and dead” assay, staining of *S. aureus* living cells using two fluorescent dyes, membrane permeable Syto 9 and membrane impermeable propidium iodide, showed that oritavancin treatment resulted in displacement of Syto9 by propidium iodide. This clearly indicates damage of the cell membrane by oritavancin resulting in increased permeability of the cell [33]. Furthermore, oritavancin induced rapid leakage of liposomes composed of lipids extracted from *S. aureus* [34], cardiolipin/POPE and POPG/POPE liposomes [35]. However, it is tempting to speculate if the ability of oritavancin to interact with glycerol backbones of phospholipids and to permeabilize those lipid vesicles at concentration where the oritavancin exert bactericidal activity toward bacteria makes the basis for its bactericidal effects. And, hence, to which extent the binding to PGN/inhibition of PGN contributes to bactericidal activity. Such multiple facets of molecular mechanism are often important to overcome bacterial resistance.

Thus, high-level oritavancin resistance has not been reported neither in the laboratory nor in clinical studies [31,36]. One significant example is the antibacterial 34 amino acid long peptidic lantibiotic nisin derived from *Lactococcus lactis*, which primarily inhibits PGN synthesis. In addition, it is established that the membrane bound PGN precursor lipid II acts as a docking moiety to attract the nisin to the bacterial membrane and to promote peptide insertion into membrane leading to permeation [37–39]. This has been reconciled by a number of studies which will be discussed in detail by E. Breukink in this issue. Briefly, nisin binds to the pyrophosphate moiety of lipid II and can adopt a stable transmembrane orientation followed by pore formation. The concentration required for disruption of anionic model membranes is much higher than the effective concentration for bacterial killing. However, anionic liposomes become more susceptible to nisin in the presence of lipid II [40] supporting the use of lipid II as a “docking moiety” to form pores and to disrupt the bacterial membrane.

In many cases it is not clear, which role the PGN may have regarding the interaction with antimicrobial peptides. Although in the following examples, interaction of AMPs with PGN has been reported, it is not obvious that the high binding affinity to PGN contributes to a determinant key event of bactericidal activity. A case in point is the human cationic polypeptide ECP (eosinophylic cationic protein), which in addition to its weak membrane disruptive capacity showed high binding affinity...
for PGN [41]. ECP is an antimicrobial RNase participating in the inflammatory processes mediated by eosinophiles. It is ~155 amino acids long containing 19 arginine residues, which results in a high PI value of 11.4 and high cationicity [42]. This further confers high affinity to negatively charged surfaces, which is considered to be important for antimicrobial activity. However, ECP induced only weak leakage of negatively charged lipid vesicles composed of either POPG or POPG/POPC mixture at its bactericidal concentration toward S. aureus [43,44]. Moreover, electron micrographs did not show any damage of the cell wall and no detectable lysis processes on S. aureus cells in the presence of ECP [41]. Also omiganan, an antimicrobial peptide derivative of the bovine cathelicidin indolicidin, which has been in clinical III phase studies, showed strong partitioning toward a PGN mesh [45]. Although fluorescence quenching studies for peptide internalization showed higher partitioning constants for anionic model membranes, the peptide failed to induce leakage of those lipid vesicles. As omiganan strongly incorporates into anionic bilayers without inducing severe membrane perturbations, it was suggested as reported for indolicidin that the peptide translocates through the membrane and acts on an intracellular target such as DNA [46]. However, it is not clear, if ECP and omiganan actually have intracellular targets.

We investigated for the first time the mode of action of a synthetic antimicrobial peptide, OP-145, developed for a screen of the human cathelicidin LL-37 using model membranes composed of both PGN and phospholipids [47]. In this study we demonstrated that OP-145 can efficiently bind to PGN of S. aureus (Fig. 4) [47] as well as to PGN of other species like Streptococcus epidermis and E. coli (Malanovic, unpublished results). Thermodynamic studies performed with liposomes

![Chemical structure of peptidoglycan.](image-url)

**Fig. 3.** Chemical structure of peptidoglycan.

![Effect of OP-145 on PGN](image-url)

**Fig. 4.** Effect of OP-145 on PGN adopted from Malanovic et al. [48]. A) Thermotropic behavior of DPPG/PGN vesicles in the presence of 2 mol% OP-145 (lower curve). The corresponding DSC thermograms of pure DPPG with and without PGN (upper curves) are shown in black/grey. B) PGN binding of OP-145 as analyzed by gel electrophoresis. Lysozyme was used as a positive control and BSA as a negative control. Lysozyme or OP-145 bound to PGN is detected in the pellet, while unbound protein remains in the supernatant.

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composed of DPPG and 0.1 wt% PGN showed that OP-145 interacts preferentially with the PGN enriched bilayer domains indicated by the disappearance of the characteristic shoulder in the thermogram (Fig. 4A). Furthermore, leakage experiments using liposomes composed of POPG in the presence and absence of 0.1 wt% PGN revealed that PGN did not affect membrane permeability of OP-145 [47]. In some other cases e.g. for peptides developed in a screen for OP-145 membrane permeability was even increased supporting the idea that PGN may assist in docking of the peptides on the bacterial surface and promoting them toward the membrane interface (unpublished, Malanovic). Thus, high binding affinity to PGN may serve as a general mechanism of the peptides entrance to the cell plasma membrane. 

All these examples show the interplay between antimicrobial peptides, PGN and plasma membrane in Gram-positive bacteria and highlight the multiple facets of AMPs that may play a role for optimal activity. Designating PGN as a mesh may be misleading, but it can be assured that the PGN sacculus of both Gram-positive and -negative bacteria is relatively porous and does not represent a permeability barrier for particles of approximately 2 nm and globular hydrophobic molecules of a maximum of 50 kDa (AMPs are between 15 and 50 amino acids, <5 kDa) that do not bind to PGN [48]. Further, PGN is not negatively charged and hence is not considered to compete significantly with the membrane for interaction with AMPs so that they can pass freely through the PGN mesh of both lineages.

3.2. Peptidoglycan as a target for innate immune recognition not interfering with AMPs activity

PGN can rather be seen as a target for innate immune recognition by a family of pattern recognition molecules e.g. peptidoglycan recognition proteins (PGRPs) [49]. These proteins are evolutionary conserved and recognize the microbes via direct local attack against indwelling pathogens and induction of the acute inflammatory response and adaptive component of immune system. Thus, in such cases binding to PGN may serve as initial event for bacterial killing. An example includes human bacterial lectin lectins of the C-type lectin family with a molecular weight of ~16 kDa. Characteristic C type lectins possess a globular structure with four functional domains: (i) carbohydrate binding domain, (ii) neck repeat region of tandem helical repeats with exposed hydrophobic residues, which allows oligomerization, (iii) transmembrane domain and (iv) cytosolic domain promoting internalization into the membrane [50]. RegIII lectins recognize the bacteria by binding to PGN carbohydrate via a Glu–Pro–Asn (EPN) tripeptide motif located in the long loop region of the protein as was demonstrated by NMR spectroscopic studies [28]. This Glu–Pro–Asn motif is required for bacterial killing, as a point mutation of Glu (E) residue in Glu–Pro–Asn motif showed reduced affinity to staphylococcal PGN and a 6 fold decrease in antimicrobial activity against Gram-positive species Listeria monocytogenes. However, the mechanism by which lectins kill bacteria is not known. Just recently it was published that RegIIIα kills bacteria by oligomerization on the membrane forming a membrane-penetrating pore [51]. Interestingly, RegIIIα lectin exhibits a point mutation in the Glu–Pro–Asn motif and bears instead a Gin–Pro–Asn (QPN) motif, which prevents binding of RegIII to PGN [28]. But, RegIIIα lectin permeabilizes membranes of Listeria monocytogenes, as shown by an increased uptake of the membrane impermeable fluorescent dye SYTOX green by measuring changes in intrinsic tryptophan residues upon contact to PC/PS liposomes as well fluorescence energy transfer between donor RegIIIα and dansyl-labelled PC/PS liposomes, it has been figured out that RegIIIα binds to negatively charged membrane phospholipids (PC/PS) but not to zwitterionic PC disrupting PC/PS membranes. In addition, electron microscopy in combination with crosslinking experiments demonstrated formation of a hexamic membrane-permeabilizing oligomeric pore with a diameter of about 100 Å in PC/PS liposomes [51]. Although it has to be emphasized that PS is not a typical bacterial lipid, but shares the negative charge with PC, it was concluded that bacterial killing resulted from uncontrolled ion efflux and subsequent osmotic lysis. All these events are not found for Gram-negative bacteria, as LPS has been identified to inhibit RegIIIα membrane permeabilization and disruption of liposomes composed of E. coli total lipid extracts or PC/PS in the presence of LPS [51]. This explains the lack of ability of RegIIIα to kill Gram-negative bacteria.

It is interesting that the ability to recognize and eliminate pathogens is versatile but still evolutionary conserved. Although proteins such as C-type lectins are bigger in size and more complex in structure than the small antimicrobial peptides, they often share the same mechanisms like membrane disruption to combat against the pathogen. One may wonder if this could be through evolution a strategy of nature to combine building blocks of known functions in domains and design more complex molecules (proteins) with increased potency to overcome the emerging resistance of microbes.

3.3. Modifications of peptidoglycan to evade innate immunity

An important strategy of pathogenic and commensal bacteria to evade innate immunity and to control autolysins involves modifications of stem peptide (amidation of D-Glu and mDAP, modification of L-alα by Gly and L-ornithine instead of meso-DAP) and glycan chains (O-acetylation of NAM, N-deacetylation of NAG and glycosylation of NAM) of PGN, documented and reviewed for diverse bacterial species elsewhere [52–54]. This widely leads to survival of the bacteria as a result of reduced recognition of the pathogen by the host receptors and hence decreased activation of the innate immune response. Modification of PGN and hence, increased antimicrobial resistance is also related to increased virulence of bacteria [55].

4. Lipoteichoic acid, an anionic polymer matrix

Most Gram-positive bacteria incorporate teichoic acid polymers into their cell envelopes that largely contribute to a bacterial negative surface charge. The basic structure of teichoic acid encompasses a soluble polymer of glycolipophosphate or ribitolphosphate repeating units that is either attached to the cytoplasmic membrane via a glycolipid anchor (lipoteichoic acid, LTA) [56] or covalently linked to N-acetylmuramic acid of PGN (wall teichoic acid, WTA) (Fig. 5). The glycolipid anchor in S. aureus is diglycosyl-1,2-diacylglycerol (DGDC) with two fatty acids of different composition [57], mostly C14:0 and branched C15:0 at the sn-2 position and C16:0:C18:0 and C20:0 at the sn-1 position of the glycerol moiety [58]. LTA deficient muα mutant of S. aureus exhibits aberrant cell growth and division and is synthetic lethal with tagO mutant defective in WTA synthesis indicating that LTA and WTA compensate for their activities and that complete loss of the anionic polymer matrix in bacterial envelopes affects the growth leading to inviability of the cells [59].

In the logarithmic phase of growth the concentration of LTA in the outer membrane layer ranges between 0.4 and 1.6% of the cell dry weight [60], which means that one LTA molecule contributes to every ninth to tenth lipid molecule [58,61]. In contrast to membrane lipids, LTA and not deacetylated LTA does not form a stable monolayer structure [62], but forms a micellar supramolecular structure in aqueous dispersion [63]. X-ray structure of staphylococcal [63] and pneumococcal LTA micelles [64] was characterized with a total diameter of 22 nm. The core made of hydrocarbon chains of the glycolipid anchor is 5 nm, which is surrounded by an 8.5 nm shell of heavily hydrated hydrophilic chains. The critical micelle concentration of LTA from several bacterial species in phosphate-buffered saline ranged from 28 to 60 μg/ml [65]. Systemic thermodynamic studies on the miscibility of LTA with DPPG revealed stable mixtures up to LTA concentrations of 20 mol% [62]. Increasing the LTA concentration in the DPPG matrix leads to an increase of the phase transition temperature indicating a stabilizing effect on lipid membranes within the head group region [58]. At higher concentrations than 20 mol% separation of both lipids

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Lipoteichoic acid (LTA)

Chemical structure of lipoteichoic acid.

Fig. 5. Chemical structure of lipoteichoic acid.

4.1. Interaction of lipoteichoic acid with AMPs

Gram-positive bacteria e.g. S. aureus contain on average 24 glycerolphosphate repeating units of 6200 g/mol weight LTA of which 70% are substituted by D-alanine [57,66]. The length of the glycerolphosphate chain varies between Gram-positive species from 15 to 50 residues. Each repeating unit of LTA contains one negative charge from the phosphate group, which potentially can attract positively charged AMPs [67,68]. Obviously, many bactericidal peptides bind with high affinity to LTA, but may in addition exhibit membrane disruptive properties like melittin, cecropin [69] and LL-37 [67] contributing to their bacterium-killing activity. Thus, it has been postulated that binding and attraction of the AMPs to LTA may initiate bacterial killing by AMPs mediating peptide’s entry into the bacteria. In other words, by building polyanionic ladder LTA and WTA may help polycationic peptides to traverse from outside to the cytoplasmic membrane.

Koprivnjak et al. [70] suggested that the killing activity of both the 14 kDa mammalian group II phospholipase A2 (gIIA PLA2) and the highly basic 45 amino acid human β-defensin 3 toward S. aureus depends on initial electrostatic interaction with WTA, as the tagO mutant lacking WTA was highly resistant to these antimicrobial peptides. Detailed analysis with highly positively charged (+12 to +17) gIIA PLA2, as deduced from recovery of [1,14C]oleate radiolabeled bacterial tagO strain, revealed that phospholipid degradation was reduced in the presence of gIIA PLA2. Moreover, the peptide was able to hydrolyze phospholipids from cell-wall depleted protoplasts from both wild type and the tagO mutant and their radiolabeled lipid pattern resembled that of the intact bacteria. As the binding of the peptide to the surface of tagO mutant was not reduced, the authors concluded that the binding of the gIIA PLA2 to WTA is important to facilitate cell wall penetration and to gain access for membrane phospholipid degradation.

Another bactericidal protein, the phospholipoglycoprotein vitellogenin, which is a major precursor of the yolk proteins in oviparous organisms, kills bacteria via binding to LTA and not via membrane disruption [68]. Results from scanning electron microscopy showed that 450 kDa vitellogenin from fish Hexagrammos otakii causes damage of the cell wall of S. aureus whole cells with the appearance of collapsed architecture, but does not induce changes in the morphology of S. aureus protoplasts, which are depleted of cell wall and hence LTA. This was also confirmed in a lysis assay, where significant reduction of OD420 indicated severe cell lysis in vitellogenin treated S. aureus whole cells, but not in cell wall depleted protoplasts. In addition, cell-wall destroying activity of vitellogenin toward S. aureus is abolished, when vitellogenin was preincubated with LTA before applying to the S. aureus cells, which concomitantly resulted in loss of antibacterial activity of the peptide. These observations suggest that the binding of vitellogenin to LTA is lethal to S. aureus. It has also been reported that the antibacterial cell-permeable peptide PBP 10, LL-37 and melittin efficiently bind to LTA inhibiting their antimicrobial activity [67]. Although it is obvious that for instance the antimicrobial activity of LL-37 is maintained by its action on the plasma membrane [71], LTA possesses inhibitory effect on its bacterium-killing activity [67]. It is most likely however that some peptides may be entrapped by LTA through an increase of peptide adsorption to the bacterial surface, which results in a decrease in local peptide concentration on the cytoplasmic membrane.

Using a fluorescence assay our group showed that OP-145, a derivative of LL-37, also binds to LTA [47]. Analyzing the thermotropic behavior of liposomes composed of DPPG and LTA characterized by two overlapping phase transitions corresponding to DPPG and DPPG/LTA domains also indicated that OP-145 interacts preferentially with DPPG/LTA domains. Further, the bilayer permeability of large unilamellar vesicles composed of POPG and LTA at a biologically relevant molar ratio
of LTA or exposure of the hydrophilic fatty acid chains of LTA micelles 
invading bacteria. Given this one can consider if the high concentration 
of bound AMPs completely saturate the bacterial membrane, one may 
consider that in the case of OP-145, binding to LTA may in fact reduce 
the total concentration of the peptide on the membrane interface, 
but not sufficiently enough to prevent significant membrane coverage 
to kill bacteria.

In addition, it has been demonstrated for resistant group B Strepto-
coccus bacteria, which via D-alanylation have decreased anionic charge 
of LTA and decreased susceptibility to AMPs e.g. LL-37, magainin 2, poly-
myxin B and colistin, that the resistance to AMPs may not necessarily 
be attributed to decreased amounts of bound peptide to bacteria, but it 
may alter conformation of the LTAs [72]. Consequently, this result in 
increased cell wall density hindering AMPs to reach the plasma mem-
brane through compact heavily coiled conformation of staphylococcal 
LTA [72]. Another example includes ß-bungarotoxin B chain, an antibac-
terial cationic polypeptide from snake venom that upon binding to LTA 
undergoes conformational changes resulting in inhibition of its active 
site that abrogates its membrane-damaging activity and inhibits its 
Bactericidal activity toward S. aureus [73]. ß-bungarotoxin is the main 
pre-synaptic phospholipase A2 neurexotoxin consisting of ~14 kDa A 
chain that shows similarities to phospholipase 2 and a 7 kDa B chain 
peptide more similar to toxin I, trypsin inhibitor and dendrotoxin. The 
ß-bungarotoxin B chain exerts membrane-damaging activity as shown 
by calcein release from liposomes composed of PG and PE but also of 
mixtures of PG and cardiolipin. The B chain exerts its damaging activity 
without involvement of A chain and because of its abundant pos-
itively charged amino acid residues it is more likely that the B chain 
displays bactericidal action via a membrane-damaging activity [74].

However, the peptide was unable to inhibit growth or induce mem-
brane permeability of S. aureus. As the membrane permeability of 
propidium iodide fluorescent dye was induced in E. coli cells treated 
with ß-bungarotoxin B chain but not in S. aureus cells, it was clear 
that components of the Gram-positive cell wall may contribute to 
these negative results. Indeed, calcein release from PG/cardiolipin 
vesicles was absolutely abolished, when the peptide was preincubat-
ed with LTA. CD spectra indicated conformational changes of the 
peptide in the presence of 4.5 mg LTA suggesting that LTA efficiently 
blocks the B-chain functional site or conformation on damaging 
membrane.

4.2. Role of lipoteichoic acid in immune response

LTA is released spontaneously into the culture medium during 
growth of Gram-positive bacteria [75] but the release can be enhanced 
upon treatment with antibiotics like penicillin [76] or after bacteriolysis 
induced by cationic peptides from leucocytes (for review see [77]). Re-
leased LTA is believed to stimulate production of inflammatory media-
tors, immune response to infection in host organism to fight the 
invading bacteria. Given this one can consider if the high concentra-
tion of LTA or exposure of the hydrophilic fatty acid chains of LTA micelles 
are initializing the activation of the signaling cascade of inflammatory 
response. Interestingly, the activation of the inflammation pathway,
5. Concluding remarks

So far limited quantitative data have been reported on the interaction of AMPs with Gram-positive cell wall components, which may be partly due to the fact that their extraction from bacterial cells is problematic and are often not pure enough to test it on a single molecule level. Moreover, it is not possible to isolate the intact PGN wall \[13\] and in addition commercially available PGN preparations often contain proteolytic enzymes. Similarly, LTA can be contaminated also with proteins and endotoxins, which may interfere with experimental interpretation. One strategy to overcome these problems is to perform experiments on live cells as extensively discussed by Castanho and co-workers \[8\]. Nevertheless, studies on membrane–mimetic systems, which definitely will become more complex in future, have revealed interesting insights into the interaction of AMPs with these components. These studies provided evidence that membrane-active cationic antimicrobial peptides on their way to cytoplasmic membranes are exposed to different interaction partners, to which they exhibit different affinities, which may reduce their effective concentration on the membrane surface (Fig. 7). Taking into account that PGN is relatively porous and freely penetrable for small molecules like AMPs and that permeability assays on simple membrane model systems were not impaired in the presence of PGN, one would assume that the role of PGN is not in entrapping AMPs but might rather act as sponge facilitating the penetration of the cell wall and in turn interaction with the phospholipid bilayer. In contrast, LTA as an anionic polymer has a strong potential to attract positively charged molecules and may act as both entrapper of AMPs but might rather act as sponge facilitating the penetration of AMPs but might rather act as sponge facilitating the penetration of AMPs but may well end up that it is a matter of concentration \[8\].

Transparency document

The Transparency document associated with this article can be found, in online version.

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