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

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ABSTRACT

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.

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 cytoplasmic membrane, interacting with the lipid matrix and subsequent permeabilization of the membrane [1–3]. Some peptides traverse the membrane and bind to intracellular targets [4,5] or exhibit, besides their antimicrobial activity, multifaceted immunomodulatory activities [6]. The mechanisms of membrane-active peptides [1,3,7] and the main characteristics of AMPs for high binding and selectivity toward microbial membranes [8] have been extensively reviewed.

It was suggested that the amino acid composition determining the physicochemical properties of the peptide in respect to charge, amphipathicity, hydrophobicity, flexibility and H-bonding capacity are key factors for their mode of action and selectivity toward microbial cells [9]. Upon contact with microbial membranes AMPs often undergo structural changes adopting defined secondary structures or oligomerize into aggregates that also account considerably for the diversity of antimicrobial mode of action [8]. Amphipathicity resulting from segregation of apolar and polar residues upon secondary structure formation favors internalization of the peptide and in turn membrane perturbation. Thereby, the presence of hydrophobic amino acids leads to stronger partitioning into membranes. Nevertheless, there is consensus that the positive charge of the peptide is essential for initial binding to the negatively charged bacterial membrane surface, which allows discrimination between bacterial and host cell membrane, and its hydrophobicity is needed for insertion into and perturbation of the membrane [10,11].

Abbreviations: AMP, antimicrobial peptide; BSA, bovine serum albumin; CD, circular dichroism; CEME, a hybrid of silk moth cecropin and bee melittin; CL, cardiolipin; DGDG, diglycosyl-1,2-diacylglycerol; DPPG, 1,2-dipamitoyl-*sn*-glycero-3-phospho-rac-glycerol; DSC, differential scanning calorimetry; ERG, ergosterol; IL-6, interleukin 6; LPS, lipopolysaccharide; LTA, lipoteichoic acid; LUV, large unilamellar vesicle; LPC, lysylphosphatidylcholine; LPG, lysylphosphatidylglycerol; LPE, lysylphosphatidylethanolamine; MLVs, multilamellar vesicles; NAM, N-acetyl glucosamine; NAG, N-acetyl muramic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PGN, peptidoglycan; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-rac-glycerol; PS, phosphatidylserine; PGRPs, peptidoglycan recognition proteins; PI, phosphatidylinositol; TNF, tumor necrosis factor; TLR, toll like receptor; WTA, wall teichoic acid.

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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 ß-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 of 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 PMAP-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

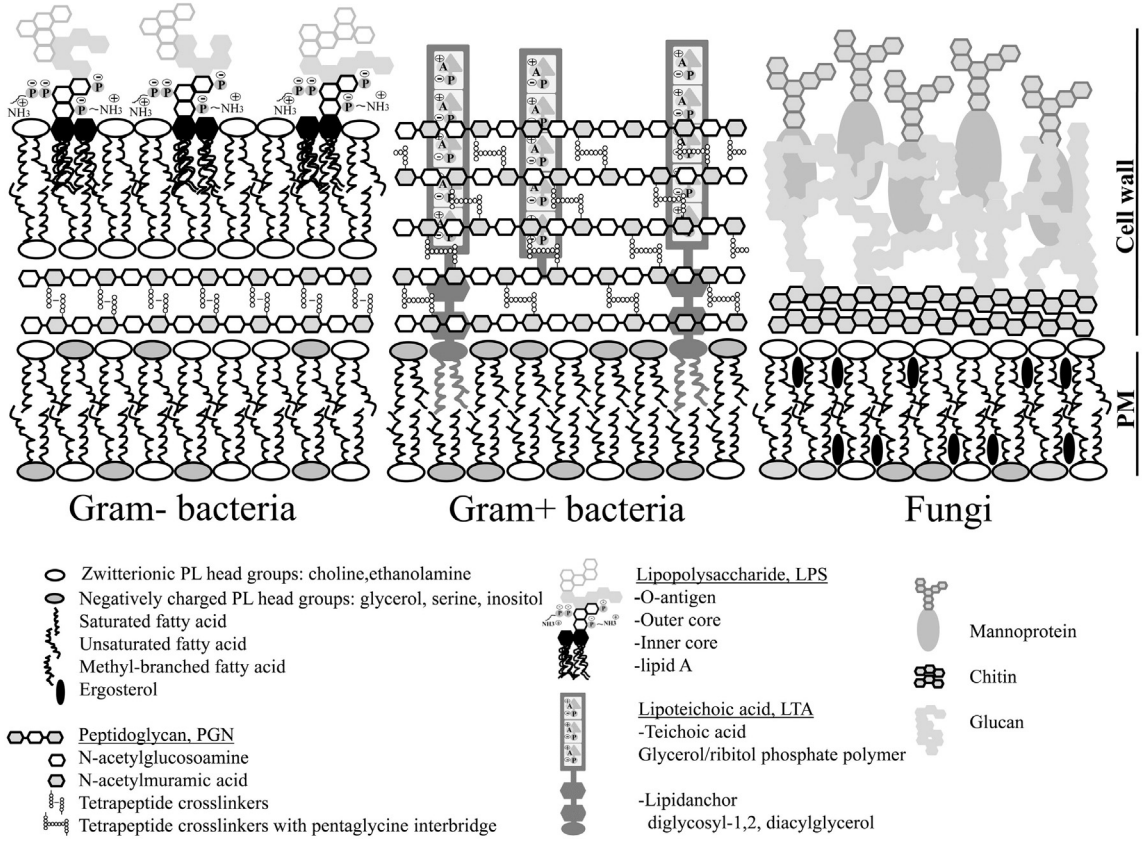

Fig. 1. Cell envelopes of various microbial families.

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