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Review

Recent progress on the application of ^{2}H solid-state NMR to probe the interaction of antimicrobial peptides with intact bacteria

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ABSTRACT

Discoveries relating to innate immunity and antimicrobial peptides (AMPs) granted Bruce Beutler and Jules Hoffmann a Nobel prize in medicine in 2011, and opened up new avenues for the development of therapies against infections, and even cancers. The mechanisms by which AMPs interact with, and ultimately disrupt, bacterial cell membranes is still, to a large extent, incompletely understood. Up until recently, this mechanism was studied using model lipid membranes that failed to reproduce the complexity of molecular interactions present in real cells comprising lipids but also membrane proteins, a cell wall containing peptidoglycan or lipopolysaccharides, and other molecules. In this review, we focus on recent attempts to study, at the molecular level, the interaction between cationic AMPs and intact bacteria, by ^{2}H solid-state NMR. Specifically-labeled lipids allow us to focus on the interaction of AMPs with the heart of the bacterial membrane, and measure the lipid order and its variation upon interaction with various peptides. We will review the important parameters to consider in such a study, and summarize the results obtained in the past 5 years on various peptides, in particular aurein 1.2, caerin 1.1, MSI-78 and CA(1-8)M(1-10). This article is part of a Special Issue entitled: Biophysics in Canada, edited by Lewis Kay, John Baenziger, Albert Berghuis and Peter Tieleman.

1. Introduction

Antimicrobial peptides (AMPs) have received a great deal of attention due to their potential to help solve the crisis of antibiotic resistance to conventional small molecule drugs [1–8]. Applications of AMPs to cancer treatment are also being explored [9–11]. A wide variety of organisms, from bacteria to humans, produce AMPs as part of their innate immune defense systems [12]. In addition to natural AMP sequences, such as caerin 1.1 and aurein 1.2 from Australian tree frogs [13,14], a number of synthetic sequences have been generated, including MSI-78 [15] and CAME [16,17]. Many AMPs exhibit a degree of specificity and can kill pathogens at concentrations that do not harm host cells. Much of this specificity is thought to relate to the cationic charge that most AMPs possess. AMPs are generally small, positively charged, have a substantial hydrophobic content and can form amphipathic structures [12]. Most AMPs are largely unstructured in solution, and fold upon membrane binding. A variety of structures have been observed in membrane-bound AMPs, including α-helical and β-sheet type structures. In this review, we focus on cationic, α-helical AMPs.

The amphipathicity of AMP structures confers a propensity to interact with lipid bilayers. And indeed, much of the research into AMP mechanisms has focussed on their interaction with membranes, either as their direct mechanism of killing via membrane permeabilization, or as a means of getting inside the cell to disrupt intracellular targets. Membrane interactions are likewise implicated in the specificity of positively charged AMPs which have stronger interactions with anionic membranes, for example bacterial or cancer cell membranes. The non-

Abbreviations: AFM, atomic force microscopy; AMP, antimicrobial peptides; CD, circular dichroism; CFU, colony forming unit; CL, cardiolipin; d_{31}-PA, deuterated palmitic acid; DHPC, dihexanoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DPC, dodecylphosphocholine; DSC, differential scanning calorimetry; LPS, lipopolysaccharides; MAS, magic-angle spinning; MIC, minimum inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGN, peptidoglycan; PE, phosphatidylethanolamine; PI, phosphatidylinositol; POPC, palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); REDOR, rotational-echo double resonance; SS-NMR, solid-state nuclear magnetic resonance

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specific nature of AMP pathogen interactions is thought to underlie the relative rarity of resistance development to AMPs as compared to conventional small molecule drugs [3,18–23]. However, there is a growing awareness that it is also critical to consider the interactions between AMPs and non-lipidic components of the target cell, such as the peptidoglycan layer or intracellular AMP targets. Consequently, many biophysicists who study AMPs are starting to include more whole cell experiments [24–27], along with the more traditional model membrane work.

2. AMP mechanisms

Mechanisms of AMP killing and growth inhibition of bacteria include both direct effects on the membrane, i.e. permeabilization, as well as targeting of intracellular components and modulation of the host cell immune system [19,28–30]. Intracellular targets of AMPs are proposed to include DNA, RNA, ribosomes, chaperone proteins, and enzymes [22,31–35].

Studies of AMPs interacting with model lipid bilayers have led researchers to suggest a variety of possible mechanisms for membrane disruption, including toroidal pores, disordered toroidal pores, carpet and barrel-stave models [22,23,36] as illustrated in Fig. 1. In toroidal pores, for example, the polar/positively charged face of the amphipathic AMP structure interacts with the headgroup of the negatively charged lipids, while the hydrophobic AMP face makes contact with the lipid acyl chains, inducing bending of the bilayer, and thus stabilizing lipid pore structures. Such defects may only need to be lined by just one or two AMPs [37,38]. Although the various membrane disruption models are frequently presented as different mechanisms, many of them can be unified by considering a phase diagram of AMP mechanism as a function of peptide concentration and lipid composition [39]. While models of membrane disruption mechanisms provide valuable insight into how AMPs function, it is clear that they are not the whole story, as there is often very poor correlation between membrane permeabilization induced by AMPs and cell growth inhibition or death [40–43]. Such observations have led many researchers to suggest that at least some AMPs kill target pathogens via a multi-hit mechanism that may well include membrane permeabilization, but with other important targets as well (e.g. [22,30,34,41,44]). In addition to intracellular AMP targets, there are a variety of non-lipid components in the cell envelope of pathogens that are likely to affect AMP activity.

3. Bacterial cell envelopes and AMP interactions

Cell envelope components that may complicate the picture of AMP-bacteria interactions derived from model lipid studies include the peptidoglycan (PGN) and lipopolysaccharide (LPS) layers, as well as membrane proteins, membrane domains, bilayer asymmetry, and the specifics of lipid composition [45–47]. Understanding how non-lipidic components affect AMP activity is critical. For instance, some cell wall constituents appear to protect bacteria from certain AMPs, while for other AMPs the opposite is true; the presence of non-lipidic cell envelope components appear to sensitize or attract AMPs to the bacteria [48–50].

The architecture of the bacterial cell envelope is different for Gram-positive and Gram-negative bacteria (Fig. 2) [51,52]. In Gram(+)

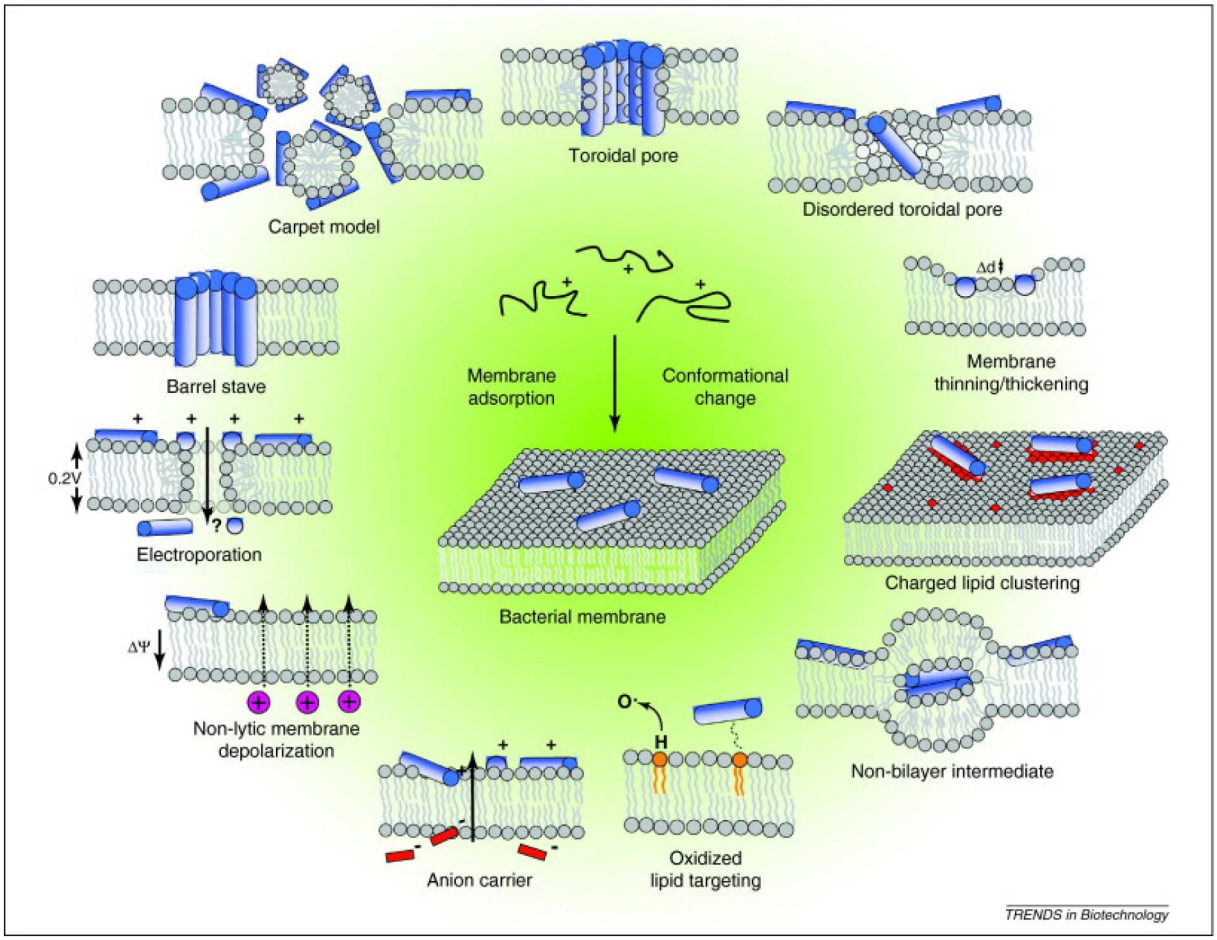

Fig. 1. Schematic of possible membrane disruption mechanisms by antimicrobial peptides.
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