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2245-Pos Board B261

Systematic Analysis of Hybrid Antimicrobial Peptides

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Antimicrobial peptides (AMPs) are part of the immune response of all classes of life and have gained attention as promising alternative treatments for infectious bacteria resistant to conventional antibiotics. AMPs kill bacteria through two known mechanisms of action. Some AMPs, such as parasin and magainin II, kill bacteria by inducing membrane permeabilization. Other AMPs, such as buforin II (BF2) and DesHDAP1, readily translocate across the membrane and interact with intracellular components including nucleic acids. In recent years, there has been increased interest in developing hybrid AMPs that combine two distinct AMPs into a single peptide. These hybrid AMPs have been shown to be more potent than their individual AMP components. To date, few studied hybrid have combined AMPs that follow different mechanisms. Here, we focus on using a variety of cellular assays and confocal imaging to characterize the activity and mechanisms of action of hybrid AMPs that combine one permeabilizing AMP (parasin or magainin II) with one translocating AMP (BF2 or DesHDAP1) in different orientations and with different linkers. We show that these hybrid AMPs are generally more potent than their individual AMP components and that the permeabilizing peptide (parasin or magainin II) dominates the mechanism of action when combined with the translocating peptide (BF2 or DesHDAP1). These observations of 16 hybrid peptides have elucidated trends that will promote the rational design of AMPs with enhanced activity.

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Aggregation vs. Fusion of Negatively Charged Lipid Bilayers Induced by Bactenecin and Magainin Derivatives

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Antimicrobial peptides (AMPs) have been studied for their future promising perspectives to overcome the arising health-treating issues of bacterial superbugs, alone or in synergy with other antibiotics. Although AMPs clinical applications are still obscured owing to their formulation challenges, salt sensitivity and stability issues, their mechanisms of action upon encountering the bacterial cell membranes is a matter of debate and require understating of both the intrinsic physicochemical properties of the membrane and crucial role of the peptide primary structure. In this study, we wanted to investigate the membrane bindings of highly selective antimicrobial bactenecin and magainin 2 variants to the negatively charged mixed POPC/POPG and POPE/POPG liposomes with the lower and higher spontaneous negative curvatures, respectively. Unlike magainin 2 derivatives, increasing the molar ratio of bactenecins to both lipid systems followed by sample turbidity and decrease in the fluorescence emission spectra of tryptophan, particularly for POPE/POPG bilayer. The electron microscopy showed the formation of stable and fragile colloidal aggregates of about 1-15 micron for the mixed liposome composed of POPE and POPG, respectively. Whereas these preliminary results were further supported by the FRET analysis using POPE/POPG system mixed with Rhod-DPPE and NBD-DOPE, liposome fusogenic properties were suggested for the membrane-lytic magainin peptide. It seems, though, both peptide variants are membrane-active and antimicrobials at the very low comparable concentrations, different antimicrobial mode of actions are expected for them. The results here turn out to dictate the design of next generation of AMPs which are less vulnerable to the membrane-associated mechanism of bacterial resistance.

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Conformations and Dynamic Transitions of a Melittin Derivative in Lipid Bilayer

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MelP5 is a lipophilic peptide with unique physical properties including the ability to create large and stable pores at low concentrations. Self-assembly into membrane spanning pores makes MelP5, systematically evolved from the primary toxic component of bee venom, a promising candidate for future applications in the pharmaceutical arena, as it offers a robust mechanism of drug delivery. Despite significant importance, little is known about the mechanism by which MelP5 remodels the lipid bilayer upon binding. Here, we demonstrate by atomic force microscope imaging that MelP5 interacts with a lipid bilayer in one of two ways: surface bound, which causes a thinning of the bilayer, or insertion into the membrane, which creates pores. Thinning of the bilayer was measured to be ~0.4 nm below the upper leaflet of the bilayer and was a prerequisite to pore formation. Pores exhibited many stable sizes, some of which were quite large. For example, approximately 20% of the pores exhibited footprint areas of 47 +/- 19.8 nm^2. Time lapse analysis demonstrated that the peptides transitioned reversibly between the membrane thinned state and pore state, yielding upper and lower bounds ($0.2 < \tau < 180$ s) on the characteristic time scale of transitions between these states.

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Dynamic Membrane Bound Structures of Melittin and Alamethicin as Revealed by Solid-State NMR and MD Simulation

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Melittin is a bee venom peptide that disrupts acidic dimyristoylphosphatidylglycerol (DMPG) bilayers as well as neutral dimyristoylphosphatidylcholine (DMPC) bilayers. ^{13}C chemical shift anisotropy of [1-^{13}C]-labeled melittin showed oscillatory shifts with the index number of residues. Analysis of the ^{13}C chemical shift oscillation properties indicated that melittin bound to a DMPG membrane adopts a bent α-helical structure with tilt angles for the N- and C-terminal helices of −32 and +30°, respectively. The transmembrane melittin in DMPG bilayers indicates that the peptide protrudes toward the C-terminal direction from the core region of the lipid bilayer to show a pseudo-transmembrane bent α-helical structure [1, 2].

The structure topology and orientation of membrane-bound antibiotic alamethicin were studied using solid state NMR spectroscopy. ^{13}C chemical shift interaction was observed for [1-^{13}C]-labeled alamethicin. The chemical shift oscillation analysis was performed with the assumption that the adjacent peptide planes form an angle of 100° or 120° when it forms α-helix or 3_{10}-helix, respectively [2, 3]. These properties lead to an oscillation of the ^{13}C chemical shift anisotropy with respect to the phase angle of the peptide plane. The chemical shift oscillation curves revealed that the N- and C-termini formed α-helix and 3_{10}-helix, and the N- and C-termini were tilted 17° and 32° to the bilayer normal, respectively.

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2249-Pos Board B265

Impact of Metallation and Oxidized Lipids on the Structure and Membrane Disruptive Effects of Host Defense Peptides Piscidin 1 and Piscidin 3

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This research investigates the membrane-interacting host defense metallopeptides piscidin 1 (P1) and piscidin 3 (P3) that are active on drug resistant bacteria. Bacterial membranes are battlegrounds for these host defense peptides (HDPs), which can leverage multiple antimicrobial strategies (e.g. copper-associated oxidative stress; structural and chemical modifications of phospholipids) to sensitize cell membranes and achieve high antimicrobial potency.

We previously showed that P1 and P3 achieve metal binding through their amino terminal Cu and Ni (ATCUN) binding motif and that their metallation enhances their antimicrobial activity. Here, we employed biophysical tools to investigate the impact of metallation and oxidized lipids (OxPL) on peptide conformation and bilayer thickness. We used UV spectroscopy to demonstrate peptide metallation and membrane binding. For high resolution