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Biophysical Mechanism of Galleria Mellonella Natural and Analoge Peptides with Bacterial Model Membranes
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Antimicrobial peptides (AMPs) are important components of the innate immune system of animals and plants. AMPs are considered promising alternatives to conventional antibiotic treatments, as they exhibit a broad spectrum activity [1]. Understanding the mechanism by which AMPs interact with membranes is fundamental for explaining their biological action. We present a study of two synthetic peptides: Gm1, a cecropine neutral D-like peptide, from Galleria mellonella with activity against Gram-positive, Gram-negative bacteria and fungi [2], and ΔGm1, a modified structure of Gm1. The aim of the modification was to evaluate the biological activity that increases the cationic amino acids.
We have studied the interaction of peptides by applying Fourier-transform infrared spectroscopy (FTIR) and Föster resonance energy transfer spectroscopy (FRET), using pholipid membranes built-up lipopolysaccharide (LPS) and dimyristoylphosphatidylglycerol (DMPG) as representative components of the outer and cytoplasmic bacterial membrane, respectively. Atomic force microscopy (AFM) was used to evaluate the effect of the peptides on the bacterial cells of P. mirabilis R45, and colony counting assay was employed to evaluate the antimicrobial activity. FTIR results showed an opposite effect on the acyl chain packing of lipids. FRET experiments confirmed the incorporation of peptides into the lipid membranes. Considerable alterations were observed in the morphology of P. mirabilis R45. The exposure of the bacteria to Gm1 leads to grooves and when exposed to ΔGm1, it induces the formation of indentations and cell debris in P. mirabilis R45. The colony counting assay showed that ΔGm1 also has biological activity.
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[2] M. Cytryńska, P. Mak, A. Zdybicka-Barabas, P. Suder, T. Jakubowicz, Purification and characterization of eight peptides from Galleria mellonella immune hemolymph, Peptides, 28 (2007) 533-546.

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Structure-Function Relationships of Antimicrobial Piscidins 1 and 3 bound to Cholesterol-Containing Lipid Membranes
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Isolated in the mast cells of hybrid-striped sea bass, piscidin 1 (p1) and piscidin 3 (p3) are antimicrobial, cationic, and amphipathic peptides that have demonstrated broad-spectrum activity against bacteria, fungi, viruses, and cancer cells. Both p1 and p3 adopt an alpha-helical structure when bound to phospholipid membranes. p1 is the more active of the two isoforms, exhibiting higher lytic activity on different bacterial strains, as well as erythrocytes. This research uses various biophysical methods to investigate the differences in the backbone structure and bilayer location of piscidin bound to different lipid bilayers of biological relevance.
To mimic the composition of human erythrocytes, a mixture of 4:1 palmitoyl-oleoyl-phosphatidylcholine (POPC):cholesterol (CHL) at pH 7.4 was used. Oriented bilayer samples prepared with 1:40 peptide:lipid were analyzed using solid-state NMR yielding 15N-1H dipolar couplings as well as 15N amide chemical shifts. These data were used to compute a high-resolution atomic-level structure of p1 and p3 bound to zwitterionic bilayers. The peptide's backbone structure is largely conserved across various bacterial and mammalian membrane mimics, suggesting that the difference in a peptide's activity on various membranes is more reliant on the peptide's side-chain and its position within the membrane.
The structural results were supplemented with dye leakage assays in order to probe the peptide's activity on lipid vesicles, mimicking bacterial and mammalian cells. These results lend insight into how specific changes in peptide's structure may result in varying activity on different membrane systems. Molecular dynamics (MD) simulations were used to predict the peptide's bilayer location and depth of insertion. Membrane thinning, an event known to precede pore formation, was shown using MD. Overall, these experiments help obtain principles to design novel antibiotic pharmaceuticals with low hemolytic effects and high lytic activity on bacteria.

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Effect of Fluorination on Membrane Interactions of an Antimicrobial Peptoid Macrocycle
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The need for new antibiotics is of increasing importance as bacteria continue to develop resistance to conventional antibacterial agents in clinical use. A promising source of potential anti-infective agents have been reported for a family of antimicrobial peptide mimics known as peptoids. However, bioavailability, and target binding affinity are key factors in determining if a bioactive compound will establish effective therapeutic efficacy in vivo. A widely used strategy in drug design is to increase metabolic stability and lipophilicity by compound fluorination. In this investigation we compare the mechanism of action of the cyclic peptoid C(3-15) and its fluorinated analog C(3-2) on model bacteria membrane systems using a high brilliance synchrotron radiation source. We have employed X-ray reflectivity (XR) on two different Langmuir monolayers mimicking the outer membrane of Gram-positive and Gram-negative bacteria composed of 1,2-dipalmitoyl-sn-glycero-3-phospho (DPPG) and the lipopolysaccharide (LPS) LipidA-Kdo2 respectively. XR reveals that although both compounds insert readily into DPPG and LPS monolayers, the fluorinated peptoid shows a greater activity than its nonfluorinated analog.

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Membrane Interactions of Antimicrobial Peptoids - Restriction of Conformational Flexibility as a Strategy to Enhance Activity
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Non-natural oligomeric mimics of antimicrobial peptides (AMPs) can be designed to display chemical moieties analogous to the active side chains of natural peptides, while their abiotic backbone provides protection from proteolytic degradation. N-substituted glycine oligomers (peptoids) are an outstanding example of potential anti-infectious agents that have evoked a significant research effort to optimize their structures. In this study, we evaluated the effect of macrocyclization on the activity of antimicrobial peptoids. Cyclization is effective strategy to restrain the peptoid molecules conformational flexibility. Here, we examined the mode of membrane interactions mode for three pairs of cyclic and linear peptoids using Langmuir monolayer constant pressure insertion assays, fluorescence microscopy, and synchrotron X-ray scattering. The outer leaflets of the outer Gram-negative and cytoplasmic Gram-positive membranes were modeled with LPS (lipid A-kdo2) and DPPG monolayers, respectively. We demonstrate that both cyclic and non-cyclic peptoids readily incorporate into the bacterial membrane mimics, causing a rapid deterioration of the structural ordering of the lipid acyl chains. We also observe that cyclic and linear peptoids differ substantially in their mechanism of action. In particular, analysis of X-ray reflectivity data shows that the cyclic peptoids penetrate into the lipid hydrophobic core to a greater extent than the corresponding linear analogues.

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Understanding the Fungicidal Activity of Lipopeptides on the Basis of their Biosurfactant Properties
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Bacillus subtilis strain QST 713 produces a wide variety of lipopeptides of the surfactin, iturin, and fengycin families. These lipopeptides are believed to be produced to attack other organisms by permeabilizing their cell membranes. In order to do that, these peptides have to induce membrane pore or leaks and at even higher concentration they may solubilize the membrane. We are interested in a better understanding of the fungicidal activity of the organism, which has been utilized for the highly effective and environmentally safe protection of crops against a variety of pathogens. We have been studying the destabilization, permeabilization and lysis of lipid vesicles by the peptides by means of isothermal titration calorimetry, the lifetime- based vesicle leakage assay, light scattering, and other methods. We find fundamentally different lytic effects of the different classes and synergistic activity on various membrane types. In this study, we have addressed the mechanism of action and surfactant like properties for the extreme lytic activity of these compounds, the interplay between the different peptides in the biologically optimized mixture, and the selectivity of the action to different lipid membranes.