and C-anchored peptides retain alpha-helical conformations. By incorporating deuterated alanines into the sequences, we were able to determine the average orientation of these peptides within mechanically aligned lipid bilayers using solid-state deuterium NMR. The bilayer length-dependent tilt of these half-anchored peptides, in DOPC, DMPC and DLPC lipid bilayer membranes, appears to be somewhat less than for WALP23. The observed average tilts range between about 1 and 6 degrees from the bilayer normal for the N-anchored and C-anchored peptides, compared to 4-8 degrees for WALP23. The intrinsically small tilt values and single anchoring region suggest that anchor residue interactions with lipid head groups may be important for the magnitude of the peptide tilt.

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Mechanisms Of Antimicrobial Peptide Action Determined Using Chemical And Collisional Quenching Assays

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Peptide-lipid interactions are pertinent to antimicrobial peptides (AMP) activity, stringency, and selectivity. The ability of AMPs to disrupt the target cell's lipid bilayer has been described using the nonspecific ''carpet'' model or using models that assume stable transbilayer pores (e.g. barrel stave pores). With the carpet model, there is a strong electrostatic and hydrophobic attraction between peptide and the lipid interfacial groups such that the peptides blanket the cell membrane. Carpet model peptides can then kill the organism by disrupting the lipid bilayer and causing loss of cellular contents. The stable pore model consists of fixed transmembrane structures that rely on an amphipathic amino acid sequence to form membrane-spanning pores. Included with these models, peptides can also induce flip-flop of bilayer lipids or form transient pores. We have developed chemical and collisional quenching assays that help determine the mode of lipid disruption associated with naturally occurring and synthetically designed AMPs. The assays require large unilamellar vesicles (LUVs) with fluorophore-attached lipid head groups in both inner and outer leaflets of the bilayer. Only peptides with stable pore-forming or detergent-like activity allow quenchers access to the inner leaflet. The combination of these quenching assays with leakage experiments and cryo-electron microscopy allows for a more complete description of the mechanism of membrane disruption by peptides.

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Investigating the Role of Proline in Buforin II Function

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Buforin II (BF2) is a 21 amino acid long antimicrobial peptide. Unlike many antimicrobial peptides that induce cell death by disrupting the cellular membrane, BF2 exhibits potent antimicrobial activity without significant membrane permeabilization. A histone derivative, BF2 is hypothesized to kill bacteria by translocating across the cell membrane and binding to nucleic acids. Its membrane-penetrating property makes it a potential model for novel drug delivery systems. Pro 11 of BF2 been shown to play an important role in membrane translocation. To investigate the role played by proline, it was replaced with alanine (P11A) or reintroduced at various locations (P11A/G7P, P11A/V12P and P11A/V15P). Changing the location of the proline residue alters the peptide's overall helicity and affects the peptide's antimicrobial activity. Lipid vesicle assays showed that an optimal amount of α-helicity appears related to translocation, as increased or decreased helicity both led to reduced translocation compared to wild type BF2. However, antimicrobial activities did not correlate clearly with translocation abilities. To better understand the antimicrobial mechanism of BF2 and the role of proline, pore formation and DNA binding were investigated. The pore-forming abilities of wild type BF2 and its proline mutants were examined with a lipid vesicle dye-leakage assay. These experiments showed that increased α-helicity correlates with peptides' increased ability to cause membrane permeabilization. A fluorescent intercalator assay was used to determine the peptides' ability to bind nucleic acids. These studies revealed that while P11A/G7P exhibits a significantly stronger DNA binding ability than wild type BF2, the other mutants have similar DNA binding abilities. Together, this data helps to explain the imperfect correlation between the peptides' respective antimicrobial activities and their abilities to translocate and sheds light on the role of proline in BF2 function.

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Clostridium perfringens α-toxin action facilitates the Perfringolysin O-cholesterol interaction

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Clostridium perfringens is a Gram-positive bacterium that causes gangrene and gastrointestinal disease in humans. These pathologies are mediated by potent extracellular protein toxins, particularly alpha-toxin (phospholipase C or PLC) and theta-toxin (perfringolysin O or PFO). While PLC hydrolyzes phosphatidylcholine and sphingomyelin, PFO forms large transmembrane pores upon binding and oligomerization on cholesterol-containing membranes.

We have shown previously that PFO binding to model membranes requires a high concentration of cholesterol and we have also shown recently that binding cholesterol molecules is necessary and sufficient to trigger all the conformational changes that effect PFO oligomerization and initiate pore formation. These results suggested that the ability of PFO to perforate the membrane of the target cells is dictated by how much cholesterol is exposed at the membrane surface.

Given that the enzymatic activity of *C. perfringens* alpha-toxin cleaves the phosphocholine head group of phosphatidylcholine,,we reasoned that PLC activity may facilitate exposure of cholesterol, thus assisting the interaction of PFO with cell membranes.

Our present studies reveal that PLC action on membrane bilayers facilitates the PFO-cholesterol interaction as evidenced by a reduction in the amount of cholesterol required in the membrane for PFO binding and pore-formation. In addition, we showed that the ability of PFO to recognize cholesterol in membranes is modulated by the structural arrangement of amino acids located at the tip of Domain 4 - a compact beta-sandwich bearing a tryptophan-rich motif. Modification of amino acids located close to a conserved residue, C459, modified the ability of PFO to bind to membranes in a cholesterol dependent manner. These studies suggest a mechanism for the concerted action of PLC and PFO during C. *perfringens* pathogenesis.

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Direct Visualization of Antibiotic-induced Pores in Phospholipid Vesicles by Cryo Electron Microscope

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Cytolytic peptides, such as Magainin, Melittin, and Alamethicin are ubiquitously present within the animal kingdom as a part of the host-defense system. Magainin-2 lyses a wide range of both gram-negative and gram-positive bacteria and a range of cancer cells. Unlike most commercial antibiotics, which interact with specific protein targets, Magainin 2 and other peptides in this class have been shown to interact directly with the lipid bilayer; therefore, it is believed that bacteria will be largely unable to develop resistance to this class of antibiotics. It is believed to initially interact with acidic lipids in the bacterial membranes through electrostatic interactions, forming an amphiphillic helix, followed by hydrophobic interactions inducing pore formation, but the issue remains controversial. A number of methods have been used to study the structure of possible pores; however, none of those methods could directly observe the pores themselves. We present a new method for studying peptide/lipid interactions, which employs cryo-EM to directly image Magainin-induced pores in phospholipid vesicles. Images of DMPC/DMPG lipid vesicles with Magainin showed both perturbed and unperturbed vesicles, while vesicles without Magainin were unperturbed**:** perturbed vesicles exhibited power spectra similar to neutron scattering experiments in the presence of Magainin. To estimate pore size, we completed a set of simulations with randomly distributed pores on spherical vesicles. The mean pore size obtained by simulation was ~83Å, which is compatible with prior neutron scattering data. In addition, since the vesicle images are projections, we performed cryo-electron tomography experiments to reconstruct the 3-D structure of the pores. For the first time, we were able to visualize antibiotic peptide-induced pores on phospholipids vesicles, and the pore size is consistent with the simulation result.

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Investigating the Bactericidal Mechanism of Three Novel Histone-Derived Antimicrobial Peptides

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Many antimicrobial peptides elicit bacterial death via cell membrane lysis. However, buforin II (BF2), a 21-amino acid peptide derived from histone H2A, is unique due to its hypothesized ability to translocate across cell membranes and interact with bacterial nucleic acids to cause cell death. Since cell-entry peptides, such as BF2, often are effective at lower concentrations than peptides that target membranes, increasing their potential as therapeutics *in vivo*. To this end, we developed three novel histone-derived antimicrobial peptides based on fragments of histones H2A (Des1), H3 (Des2) and H4 (Des3). These histones were previously found to exhibit translocation behavior. The designed peptides' antimicrobial properties were verified using a radial diffusion assay. In this assay, BF2 exhibited the greatest antimicrobial activity, followed by Des1, Des3 and Des2, respectively. We also measured the absorbance of