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**The Antimicrobial Peptide Analog Magainin-H2 Induces Changes in the Phase Behavior and Lateral Organization of Supported Bilayers During Liquid-Crystalline/Gel Phase Coexistence**

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Antimicrobial peptides (AMPs) disrupts membrane function in bacterial cells. The mechanism for this is still the subject of diverse studies. It is generally believed that AMPs enriched in cationic residues are attracted to the anionic bacterial membrane through electrostatic interactions, but the mode of membrane disruption appears to vary among AMPs. For some types of AMPs, for example Magainin, protegrins, alamethicin and melittin, the adhesion to the bilayer generates a series of configurational changes leading to pore formation. After binding, these peptides accumulate on the cell surface, adopting an alpha helical configuration. This accumulation leads to membrane thinning, followed by the translocation of the peptide across the membrane and the formation of a pore induced by aggregation. We are interested in exploring how the physical state of the membrane and its lateral organization is influenced by AMP binding. In order to accomplish this we visualize DMPC supported bilayers close to a liquid-crystalline/gel phase transition temperature by atomic force microscopy in real time as Magainin-H2 (Mag-H2), a magainin analog that binds to zwitterionic membranes, is added. Close to the phase transition temperature of 24°C coexisting liquid-crystalline/gel phase domains are observed with a height difference of approximately 1.5 nm. After addition of the peptide we observe morphological changes in the domain structure and an increase in the height difference between the two phases, eventually leading to amorphous hole formation. Laurdan generalized polarization measurements indicate that the presence of Mag-H2 increases the phase transition temperature of DMPC, indicating an increase in membrane packing close to the phase transition temperature. The results are relevant in pointing to a mechanism for membrane perturbation by Mag'H2 through changes in the lateral organization of the membrane.

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**Molecular Basis for Membrane Pore Formation by Bax Protein Carboxyl Terminus**

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Bax protein plays a key role in mitochondrial membrane permeabilization and cytochrome *c* release upon apoptosis. There is compelling evidence that the C-terminal peptide of Bax has strong capability of forming pores in lipid membranes. Moreover, our recent data have indicated that the intracellularly expressed peptide translocates to the mitochondria and exerts lethal effect on cancer cells, identifying the C-terminal peptide of Bax as a potential cytotoxic agent. We have shown that the C-terminal 20-amino acid stretch of Bax (BaxC-KK; VTIFVAGVLTASLTIWKKMG), as well as two mutants where the two lysines are replaced with glutamate (BaxC-EE) or leucine (BaxC-LL), form relatively large pores in lipid membranes. The pore structure is analyzed by polarized Fourier transform infrared, circular dichroism, and fluorescence experiments on the peptides reconstituted in phospholipid membranes. The peptides assume an $\alpha/\beta$-type secondary structure within membranes. Both $\beta$-strands and $\alpha$-helices are significantly (by 30-60 degrees) tilted relative to the membrane normal. The tryptophan residue embeds into zwitterionic membranes at 8-9 Å from membrane center. Membrane anionic charge causes a deeper insertion of tryptophan for BaxC-KK and BaxC-LL but not BaxC-EE. Combined with pore stoichiometry determined based on the kinetics of calcein release from lipid vesicles, these structural constraints allow construction of a model of the pore where eight peptide molecules form an "$\alpha/\beta$-ring" structure with pore inner diameter of 20-22 Å. These results identify a strong membranotropic activity of Bax C-terminus and propose a new mechanism by which peptides can efficiently perforate cell membranes. Knowledge on the pore forming mechanism of the peptide may facilitate development of peptide-based therapies to kill cancer or other detrimental cells such as bacteria or fungi.

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**Structural Studies of Neuropeptide Y and NPY-[18-36] in the Presence of Neuronal Lipid Membrane Mimics**

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Neuropeptide Y (NPY), a 36-residue long polypeptide and one of the most abundant neuropeptides found in the mammalian nervous system, activates G-protein coupled receptors. As a neurotransmitter, it helps regulate metabolism and the cardiovascular system. It has also been shown to have antimicrobial properties. To better understand multiple functions of NPY, it is important to characterize its structural features under physiologically-relevant conditions. Previous studies have shown that NPY interacts strongly with phospholipids that mimic neuronal cell membranes. In this regard, we recently used CD and solid-state NMR to show that 2:1PC:PS (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine/ 1-palmitoyl 2-oleoyl phosphatidylserine), a suitable lipid mimic for neuronal membranes, could be used for high-resolution structural studies of NPY and antagonist NPY-[18-36].

In this research, we have used solid-state NMR more extensively on NPY and NPY-[18-36] to investigate their high-resolution structures and dynamics in the presence of 2:1 POPC/POPS bilayers. Two-dimensional heteronuclear correlation solid-state NMR spectra have been collected on 15N-amide backbone labeled peptides in the presence of aligned lipid bilayers. 15N chemical shifts and 15N-1H dipolar couplings have been used to obtain the topological orientation and preliminary backbone structures of the peptides. The data indicate that the alpha-helical portion of the peptides adopt an in-plane bilayer orientation and diffusive rapidly in the plane of the membrane. To investigate the membrane-binding and membrane-disrupting abilities of NPY and NPY-[18-36], we performed CD and dye leakage experiments on samples containing vesicles of various lipid compositions and different peptide concentrations. We will discuss the results in terms of the peptide's ability to disrupt lipid bilayers mimicking mammalian and microbial cells. Overall, these studies provide valuable knowledge to help elucidate the molecular basis of NPY's multiple functions in the human body.

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**Synergistic Membrane Permeation by Antifungal Lipopeptides and Detergents**

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The fungicidal activity of Bacillus subtilis QST713, based mainly on the production of cyclic lipopeptides of the fengycin (FEs), surfactin (SFs), and Iturin (Its) families, has been utilized for the highly effective and environmentally safe protection of crops against a variety of pathogens. There has been evidence that the membrane perturbation by these these and other antimicrobial peptides follow a detergent-like mechanism. We have used the lifetime-based membrane leakage assay (Soft Matter, 2009, 5, 2849-51) to study the concerted action of the lipopeptides and detergents to induce leakage of liposomes made from POPC or yeast lipid extract. The results provide further insight into similarities and differences between detergent and peptide effects on membranes.

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**From Pores to Micelles - A Peptide-Membrane Study**

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Antimicrobial peptides are known to interact strongly with negatively charged lipid membranes, initially by peripheral insertion of the peptide into the bilayer, which for some antimicrobial peptides will be followed by pore formation, and successive solubilization of the membranes resulting in mixed peptide-lipid micelles. We have investigated the mode of action of the antimicrobial peptide mastoparan-X using isothermal titration calorimetry (ITC) and cryo-transmission electron microscopy (cryo-TEM). The results show that mastoparan-X induces a range of structural transitions of POPC/POPG (3:1) lipid membranes at different peptide/lipid ratios. It has been established that ITC can be used as a fast method for localizing membrane transitions and when combined with DLS and cryo-TEM can elucidate structural changes, including the threshold for pore formation and micellation. Cryo-TEM was employed to confirm the structural changes associated with the thermodynamic transitions found by ITC. The pore-formation process has furthermore been investigated in detail and the thermodynamic parameters of pore formation have been characterized using a system-specific temperature where the enthalpy of peptide partitioning becomes zero ($T_{zero}$). This allows for an exclusive study of the pore-formation process. The use of ITC to find $T_{zero}$ allows for characterization of the thermodynamic parameters of secondary processes on lipid membranes.