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Membrane-Active Peptides and Toxins I

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The Interaction Between the Antimicrobial Peptide K-Hya1 and Model Membranes: Distinct Action in Neutral or Negatively Charged Bilayers

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Antimicrobial peptides (AMPs) are important molecules for the innate immune defense system of plants and animals. The antimicrobial peptide Hylina1 (Hya1) was isolated from arboreal South African frog Hypsiboas albopunctatus. In this work, we investigate the interaction of an analogue peptide, K-Hya1 (KIFGAIWPLALGALKNLIK-NH2) with model membranes composed of DPPC (dipalmitoyl phosphatidylcholine), DPPG dipalmitoyl phosphatidylglycerol) and DPPC:DPPG 1:1 or of POPC (palmitoyl oleoyl phosphatidylcholine) and POPC:POPG (palmitoyl oleoyl phosphatidylglycerol) with different techniques. Differential Scanning Calorimetry (DSC) profiles show distinct environments in the bilayer with anionic lipids, suggesting a disturbed region due to peptide adsorption, and an unaltered region. On the other hand, in neutral membranes an average perturbation is observed, which suggests a superficial interaction. Steady-state fluorescence spectroscopy of the intrinsic Trp residue shows a deeper insertion of this residue into charged bilayers as compared with neutral membranes. Dye-leakage experiments show that membrane charge also modulates the kinetics of membrane permeabilization, which is much faster for charged bilayers. Optical microscopy of giant unilamellar vesicles (GUVs) in the fluid phase revealed a different mechanism of action of the peptide in the presence or absence of negatively charged lipids. K-Hya1 induces small perturbations in POPC vesicles, causing membrane permeabilization without morphological changes. On the other hand, the peptide induces permeabilization accompanied by a large increase in surface area of POPC:POPG vesicles, suggesting the opening of several pores in the bilayer. Taken together, the results clearly show a peptide-bilayer interaction modulated by the presence of negatively charged lipids. Data can be interpreted as a different orientation of the peptide in the bilayer: parallel to the surface in neutral membranes and stable and crossed in anionic membranes.

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Cholesterol Incorporation in Membranes Attenuates the Disruption Ability of Antimicrobial Peptide Protegrin-1

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The ability of antimicrobial peptides (AMPs) to target and lyse the harmful microbial membrane over that of a host's is a unique characteristic making these innate immune effectors promising candidates to fill the growing therapeutic void resulting from antibiotic drug resistance. This discriminatory behavior is believed to strongly depend on the chemical and structural properties of the lipids that comprise the cell membrane. For instance, the selectivity of AMPs can be based on the electrostatic attraction of these predominately cationic peptides for the bacterial membrane surface heavily populated with negatively charged lipid components. We have previously shown that zwitterionic dimyristoylphosphatidylcholine (DMPC) bilayers display concentration-dependent structural transformations induced by protegrin-1 (PG-1) that progress from fingerlike instabilities at bilayer edges, to the formation of pores, and finally to a network of wormlike micelles. The increasing degree of membrane structural disruption in charge-neutral membranes demonstrates that a more complex interaction than that suggested by a simple electrostatic argument is needed to explain AMP selectivity. We propose that in addition to an electrostatic consideration, specific membrane compositional differences between host and pathogen tunes AMP activity to selectively disrupt microbial membranes rather than those of the host. We have tailored our investigations to utilize membrane components which eukaryotes and prokaryotes contain in drastically different proportions, specifically the presence and absence of cholesterol respectively. In these results we have employed a variety of biophysical techniques to elucidate how increasing cholesterol content in solid-supported DMPC bilayers retards the ability of PG-1 to induce membrane disruption. Atomic force microscopy was used to assess the propensity for pore formation, while neutron reflectivity and oriented circular dichroism studies were advantageous in providing molecular level detail on the location and orientation of PG-1 with respect to the membrane.

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Lipid Extracting Effect of Daptomycin Correlated to its Action on Bacterial Cell Membranes

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Daptomycin is the first approved member of a new structural class of antibiotics, the cyclic lipopeptides. The peptide interacts with the lipid matrix of cell membranes, inducing membrane permeability to ions, but its molecular mechanism has been a puzzle. Unlike the ubiquitous membrane-acting host-defense antimicrobial peptides, daptomycin does not induce pores in the cell membranes. Thus how it affects the membrane permeability to ions is unclear. We studied its interaction with giant unilamellar vesicles (GUVs) and discovered a lipid-extracting phenomenon that correlates with the direct action of daptomycin on bacteria membranes observed in a recent fluorescence-microscopic study. Lipid extraction occurred only when the GUV lipid composition contained phosphatidylglycerol (PG) and in the presence of Ca^{2+} ions. Lipid extraction did not happen when Ca^{2+} was replaced by other divalent ions such as Mg^{2+} or PG was substituted by cardiolipin, another main component in bacterial membrane. With daptomycin, DOPG and Ca^{2+}, we found Ca^{2+} permeating into the GUV, while a content dye, Texas red dextran, did not leak out. This result suggests that daptomycin and Ca^{2+} do not form pores in the membrane of DOPG-contained GUV, but cause leakage of ions. Furthermore, the lipid extraction effect occurred only when the peptide-lipid ratio exceeded a threshold value. This threshold value explains the order of the magnitude of the minimum inhibitory concentration of daptomycin. Previously tea catechin, Epigallocatechin gallate (EGCg) and human islet amyloid polypeptide (hIAPP) have also been noted for their lipid extracting effects and their bactericidal or cytotoxic activities. The correlated membrane activities of EGCg, hIAPP and daptomycin suggest a causal relationship between the lipid extracting effect and membrane permeability to ions, which represents a so-far unrecognized antibacterial mechanism.

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Understanding How the Antimicrobial Peptide Thanatin Interacts with the Lipid Bilayer of Cell Walls Using Model Membranes

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Due to a misuse of classical antibiotics, multi-drug resistant bacteria are spreading worldwide, increasing troubles related to the treatment of infectious diseases. Antimicrobial peptides are among the most suitable candidates to solve this problem since they induce little or no resistance and are active against a large spectrum of pathogens. Hundreds have been isolated from prokaryotes, plants and animals. In this study, we focus on the peptide thanatin which is naturally produced by the spined soldier bug, *Podisus Maculiventris*. Its activity against multiple Gram negative and Gram positive bacteria and fungi has already been reported (Fehlbaum, P., 1996). However, the mechanism underlying its bactericidal effect is still misunderstood.

Since actual cell walls are not suited for most spectroscopic techniques due to their complexity, various mixtures of lipids were used to model the membranes of eukaryotic cells and bacteria. The effects of thanatin on multi- and unilamellar lipid vesicles were observed. ^{31}P solid-state NMR allows the determination of the lipid head group dynamics and the vesicle morphology in the absence and presence of peptide. Dynamic light scattering and UV-vis spectroscopy were used to measure the aggregation of the vesicles induced by thanatin under different conditions. These results are in good agreement with the behavior of bacteria treated with thanatin *in vivo*. Complementary results on the order of the lipid acyl chains and the secondary structure of the peptide are obtained with infrared spectroscopy.

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A Novel Functional Class of Pore-Forming Peptides

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Peptides that self-assemble into membrane spanning pores, at low concentration, to allow the passage of macromolecules would be beneficial in multiple areas in biotechnology, including biosensor design and drug delivery. However, there are few, if any, natural or designed peptides have this property. Here we show that the 26-residue peptide ''MelP5'', a gain-of-function variant of the bee venom lytic peptide melittin, identified in a high-throughput screen for small molecule leakage, enables the passage of macromolecules across bilayers. In surface supported bilayers, MelP5 forms unusually high conductance, equilibrium pores at low peptide concentration, and also increases the

capacitance of the supported bilayers, suggesting that it forms large pores that affect a significant fraction of the membrane surface. The increase in bilayer conductance due to MelP5 is much higher than the decrease due to either melittin or by the potent equilibrium pore-forming peptide, alamethicin. This result prompted us to develop two novel assays for macromolecule leakage from vesicles and use them to characterize MelP5, melittin and alamethicin. Under conditions where osmotic lysis does not occur, MelP5 allows the passage of macromolecules across vesicle membranes at peptide to lipid ratios as low as 1:100. Neither the melittin nor alamethicin release macromolecules significantly under these conditions. Therefore, the macromolecule-sized, equilibrium pores forms by MelP5 are unique, and MelP5 appears to belong to a novel functional class of peptide with many potential biotechnological applications.

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Peptides with the Same Composition, Hydrophobicity, and Hydrophobic Moment Bind to Phospholipid Bilayers with Different Affinities

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We investigated the dependence of membrane binding on amino acid sequence for a series of amphipathic peptides derived from δ-lysin. δ-Lysin is a 26 amino acid, N-terminally formylated, hemolytic peptide that forms an amphipathic α-helix bound at membrane-water interfaces. A shortened peptide, lysette, was derived from δ-lysin by deletion of the four N-terminal amino acid residues. Five variants of lysette were synthesized by altering the amino acid sequence such that the overall hydrophobic moment remained essentially the same for all peptides. Peptide-lipid equilibrium dissociation constants and helicities of peptides bound to zwitterionic lipid vesicles were determined by stopped-flowfluorescence and circular dichroism. We found that binding to phosphatidylcholine bilayers was a function of the helicity of the bound peptide alone and independent of the a priori hydrophobic moment or the ability to form intramolecular salt bridges. Molecular dynamics (MD) simulations on two of the peptides suggest that sequence determines the insertion depth into the bilayer. However, deeper bilayer insertion observed in the MD simulations is not synonymous with slow peptide desorption from the bilayer, as determined experimentally. We also found a systematic deviation of the experimentally determined dissociation constant and that predicted by the Wimley-White interfacial hydrophobicity scale. The reason for the discrepancy remains unresolved but appears to correlate with a predominance of isoleucine over leucine residues in the lysette family of peptides.

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Spectroscopic Investigations of Synthetic Amphiphilic Peptides in Interactions with Model Membranes

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A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. These short cationic peptides are being considered as a new generation of antibiotics and represent great hopes against multiresistant bacteria which are an important clinical problem. Despite their diversity, antimicrobial peptides generally share common characteristics such as a short length of amino acids, a positive charge and an amphiphilic character. Also, it is important to note that the main target of antimicrobial peptides is the membrane(s) of pathogens. We have previously shown that a non-natural peptide composed of 14 residues (10 leucines and 4 phenylalanines modified with a crown ether) has a helical secondary structure, and is able to disrupt lipid bilayers but is not selective towards bacterial membranes. To gain specificity against negatively charged membranes, several leucines of this 14-mer have been substituted by positively charged residues (lysine, arginine, histidine). In addition, we have compared the results with those obtained with peptides substituted with negatively charged residues. Solid-state NMR experiments performed in model membranes and lipids oriented between glass plates were used to better characterize the mode of action of the charged peptides. We have also performed experiments by using the magic angle spinning technique to determine if the analogues are able to induce the segregation of anionic phospholipids. Complementary results have also been obtained by infrared and fluorescence spectroscopy. The results indicate significant differences in the membrane interactions of cationic and anionic peptides, confirming the importance of electrostatic interactions.

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Investigating the Activities of Gramicidin a in the Presence of Ionic Liquids(Ils)

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Ionic liquids(ILs) have been considered as eco-friendly solvent that can replace organic solvent due to their special properties including non-volatility, non-explosiveness, high return yield, and high manipulability. Although ILs are proven as green solvent, the micro-spills into aquatic environment are inevitable. Previous researches have already shown the toxicity of ILs to diverse organisms including bacteria and zebra fish. Our previous research has also shown that the toxicity of ILs to biological organisms may be attributed to the molecular interactions of ILs with cell membranes. However, the effects of ILs on membrane proteins have not been studied yet. The membrane proteins that play important roles in the homeostasis of biological organisms are very susceptible to the perturbation of membranes. It is important to investigate the activities of membrane proteins in the presence of ILs. In this study, we chose gramicidin A(gA) as a model membrane protein. The structure of a membrane is essential to maintain the gA dimers, thus the perturbation of membrane structure may influence the dwell time of gA dimers, resulting in the change of ion current across the membrane; gAs need to be dimerized to permeate ions through the membrane. In order to study the activities of gAs in the presence of ILs, we measured the effects of ILs on gAs using two methods: fluorescence and electrical assay. The effects of ILs increase with regard to the length of alkyl chain and the concentration of ILs. In addition, the flux of monovalent cations is hindered by the positive charge of ionic liquids. Our work suggests that ILs perturb the structure of cell membranes, giving rise to the functional changes of membrane proteins as well. We believe that our research help to design more environment friendly ILs.

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The Amantadine-Resistant S31N Mutant of Influenza a Virus M2 Protein Stably Forms a Dimer on the Living Cells

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The integral matrix protein M2 of influenza A virus has been proposed to form a pH-gated proton channel and is the target of the antiviral drug amantadine hydrochloride (Am). Am-resistant viruses (H3N2) isolated from patients during 2005−06 in the United States were screened, of which more than 90% contained the S31N mutation in the M2 protein[1]. By using fluorescence resonance energy transfer (FRET), we have demonstrated that the full-length wild-type M2 proteins (H3N2) on living cells formed a dimer at neutral pH, which was converted to a tetramer at acidic pH. In the present study, we examined the oligomeric state of the S31N mutant. The mutant exhibited a dimeric FRET signal independent of pH, whereas the S31A mutant displayed a tetrameric signal at acid pH similarly to the wild type, indicating that the S31N mutant stably formed dimers and Asn at the 31st position disturbs the arrangement of helices to inhibit tetramerization. We also found that Am did not affect both the channel activity and the oligomeric state, whereas cholesterol removal reduced the activity. These results indicate that the interaction with cholesterol allows the dimer to conduct protons and the resistance could be attributed to a change of the arrangement of helices interfering Am binding.

Reference

[1] *JAMA* (2006) **295**; 891.

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Using Spheroplasts to Study Peptide Interactions with Cell Membranes

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Cytoplasmic membranes remain intact if bacteria cells are stripped off the outer cell wall and the peptidoglycan-they are called spheroplasts or protoplasts. This allows us to study the interaction of membrane-active antibiotics directly with the cytoplasmic membranes of bacterial. For our purpose, we want giant spheroplasts in order to apply the aspiration techniques. The technique of aspiration serves two purposes: one is to apply tension to the membrane and another is to measure the membrane area changes. The measurement of the membrane area change by tension or peptide binding or any structural event is the best physical quantitative description for the state of the membrane. We believe that these