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.

2351-Pos Board B321

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

Aram J. Krauson, William C. Wimley.
Tulane University, New Orleans, LA, USA.

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.

2352-Pos Board B322

Investigating the Role of Proline in Buforin II Function

Yang Xie, Natalya P. Maharaj, Eleanor Fleming, **Donald E. Elmore**.
Wellesley College, Wellesley, MA, USA.

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.

2353-Pos Board B323

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

Paul C. Moe, Alejandro P. Heuck.
University of Massachusetts, Amherst, MA, USA.

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

Mikyung Han[1], Yuan Mei[2], Htet Khant[1], Steven J. Ludtke[1].
[1]Baylor College of Medicine, Houston, TX, USA, [2]Rice University, Houston, TX, USA.

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.

2355-Pos Board B325

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

Anna T. Lee, **Hoi See Tsao**, Natalya P. Maharaj, Donald E. Elmore.
Wellesley College, Wellesley, MA, USA.

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

bacterial cells incubated with these peptides. Based on the similarity between the absorbance versus time trend for the designed peptides with other DNA-binding antimicrobial peptides, such as BF2 and indolicidin, molecular dynamics simulations were used to model the peptides' interactions with nucleic acids. MM-GBSA analyses of the simulations were used to calculate DNA binding energies of individual peptide residues. We used these analyses to create mutant versions of the designed peptides that were predicted to have altered DNA binding. Experimental measurements of the DNA binding and antimicrobial properties of these variants will help us determine whether nucleic acid interactions are important in the bactericidal mechanism of the designed peptides. Ongoing work on the designed peptides is aimed at investigating their translocation behavior *in vitro* with lipid vesicles and i*n vivo* with bacterial cells using confocal microscopy and fluorescently tagged peptides.

2356-Pos Board B326

Investigating the Effects of Acylated Lactoferricin Peptides on the Properties of Lipid Bilayers Using Gramicidin A Channels as Probes

Shemille A. Collingwood[1], Olaf S. Andersen[1], Faith Hurd[2], Denise V. Greathouse[2].
[1]Weill Medical College of Cornell University, New York, NY, USA, [2]University of Arkansas, Fayetteville, AR, USA.

Lactoferricin is an anti-bacterial peptide that is released from the iron-binding glycoprotein lactoferrin through enzymatic cleavage by pepsin. Lactoferricin is found mainly in milk and secreted fluids such as tears, saliva, bronchial mucus and seminal fluid, and it plays an important and multi-functional role in host defense, as it is part of the body's primary defense against bacteria, fungi, protozoa and viruses. It also has antitumor and immunological effects. Previous studies show that lactoferricin may inhibit bacterial growth by two different mechanisms: by sequestering the iron necessary for bacterial nutrition; and by adsorbing to bacterial plasma membranes, which may disrupt the membrane barrier properties or some other membrane function. Lactoferricin indeed permeabilizes bacterial membranes, but it remains unclear whether this is the primary mechanism by which it exerts its anti-bacterial activity. We therefore explored whether lactoferricin analogues could alter other bilayer properties, using gramicidin A (gA) channels of different lengths as probes. Specifically, could the lactoferricins alter lipid bilayer elasticity or intrinsic curvature. We tested two amino acylated lactoferricin derivatives, NC2-LfB-1MeTrp5 and NC4-LfB-1MeTrp5 (with the sequences Ac-R-R-W-Q-MeW-R-NH_2 and Bu-R-R-W-Q-MeW-R-NH_2). Both compounds increase gA channel appearance rates and lifetimes, meaning that they decrease bilayer stiffness, at concentrations (1-10 μM) where they do not cause a breakdown of lipid bilayer barrier properties. Because they had similar effects on the lifetimes of the long and short channels, we conclude that the lactoferricins alter lipid intrinsic curvature.

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On the Role of Helix-Disrupting Amino Acid Residues in Supporting the Activity of Helical Antimicrobial Peptides Isolated from Australian Tree Frogs

Ruthven N. Lewis[1], Katalin V. Korpany[1], Maria Y. Lee[1], Frances Separovic[2], Colin T. Mant[3], Robert S. Hodges[3], Ronald N. McElhaney[1].
[1]University of Alberta, Edmonton, AB, Canada, [2]University of Melbourne, Melbourne, Australia, [3]University of Colorado, Denver, CO, USA.

The peptides Aurein 1.2, Citropin 1.1, Maculatin 1.1 and Caerin 1.1 are members of four structurally related families of antimicrobial peptides produced in the skin secretions of Australian tree frogs. Although largely unstructured in aqueous solution, these peptides exhibit a high propensity for folding into amphipathic alpha helices when partitioned into lipid bilayers or dissolved in membrane mimetic media. Some of the distinguishing features of these families of antimicrobial peptides are that they usually form alpha helical structures with large hydrophobic surfaces (hydrophobic angle ~200-240°), and the amino acid sequences of many of the larger members (i.e. those with sequence lengths >~18 aa residues) usually contain significant amounts of helix-disrupting residues such as glycine and proline, the presence of which seems to be essential for the retention of antimicrobial activity. These helix-disrupting amino acid residues seem to be preferentially located in the C-terminal regions of the peptide where they tend to disrupt the break up the helical rod into two or more helical sections separated by disordered ''flexible hinge'' regions. The role of these ''flexible hinges'' has been the subject of considerable study and speculation. Our studies show that because of their large hydrophobic surfaces, they form helices with a high propensity for self association in aqueous media, and this property markedly diminishes the aqueous monomeric solubility of such peptides. Our results suggest the helix-disrupting amino acid residues may be essential for maintaining the aqueous solubility of these antimicrobial peptides

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Fine-Tuning of Acyl-Lysine Antimicrobial Peptide Mimics

Andrey Ivankin[1], Hadar Sarig[2], Amram Mor[2], David Gidalevitz[1].
[1]Illinois Institute of Technology, Chicago, IL, USA, [2]Technion-Israel Institute of Technology, Haifa, Israel.

Non-natural mimics of antimicrobial peptides are excellent candidates for anti-infectious agents due to their stability towards enzymatic degradation and broad adjustability of physicochemical properties. Acyl-lysine oligomers have demonstrated capability to be fine-tuned to high antimicrobial activity and negligible toxicity towards human cells. In this work we examine the effect of amino group or a double bond on the N-terminal acyl on interactions of the oligomer with model lipid monolayers using the liquid surface X-ray scattering techniques of X-ray reflectivity and grazing incidence X-ray diffraction. Lipid monolayer formed at the air-liquid interface mimics the membrane interface where antimicrobial peptides approach the outer leaflet of a target cell membrane. Simplified model of an outer leaflet of a bacterial membrane was represented either with DPPG or Lipid A, while mammalian cell membrane was mimicked with zwitterionic DPPC. The peptides were subsequently injected into the aqueous subphase and allowed to interact with the lipid layer. In addition to X-ray experiments, the lipid phase morphology before and after peptide mimics insertion for each lipid film was visualized by epifluorescence microscopy. Significantly higher insertion of the peptide mimics into anionic rather than zwitterionic lipid monolayers strongly supports the activity trends observed in previously reported antimicrobial and hemolytic assays. Although removing of the double bond notably increases peptide's selectivity and introduction of the amino group increases peptide's potency against bacteria, both of the modifications substantially increase MIC of the oligomers.

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Investigation of Antimicrobial and Lipid Perturbing Properties of Lactoferrin Peptides

Laura A. Bradney, Vitaly V. Vostrikov, Denise V. Greathouse.
University of Arkansas, Fayetteville, AR, USA.

An increase in bacterial resistance to conventional antibiotics has led to an intense search for alternative treatments. Lactoferricin B (**FKCRRWQWRMKKLGAPSITCVRRAF**), a peptide with potent broad-spectrum antimicrobial activity, is released by pepsin from bovine lactoferrin. A smaller amidated peptide, (LfB6; **RRWQWR**-NH_2), has been identified as having the core antimicrobial activity (Tomita et al. (1994) *Acta Paediatr Jpn.* 36:585-91). The exact mechanism by which antimicrobial peptides interact with bacterial cell membranes is not well understood, but it is proposed to depend on lipid composition. In contrast to mammalian membranes which are comprised primarily of neutral lipids, bacterial membranes contain a significant (~20-25%) fraction of negatively charged lipids. In the case of LfB6, the presence of two tryptophans (W; Trp) and three arginines (R) are thought to promote selective interaction with bacterial cell membranes. Recently, we have shown that the antimicrobial activity of LfB6 peptides is increased by N-acylation and Trp-methylation (Greathouse et al. (2008) *J. Pept. Sci.* 14:1103-1110).

To ascertain whether LfB peptides perturb lipids with negatively charged head groups, macroscopically aligned bilayers composed of lipids to mimic bacterial cell membranes have been prepared in the absence and presence of peptide. The samples are composed of neutral (POPE) and anionic (POPG) lipids (3:1), containing either s*n-1* chain perdeuterated POPE-d_{31} *or* POPG-d_{31}. The effects of LfB6 and amino acylated LfB peptides on lipid dynamics are being investigated by solid-state deuterium NMR spectroscopy and differential scanning calorimetry. The ^{2}H NMR spectra reveal that the addition of LfB6 results in slight but specific changes in the outer quadrupolar splittings, which result from the methylene groups closest to the lipid head groups. Antimicrobial assays against *S. aureus* and *E. coli* demonstrate that the activity of N-acylated LfB peptides increases with acyl chain length.

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Interaction And Unfolding Of A Model Exchangeable Apolipoprotein, ApolpIII, At Lipid Model Membranes

Edgar E. Kooijman[1], David Vaknin[2], Wei Bu[2], Leela Joshi[1], Shin-Woong Kang[1], Koert N.J. Burger[3], Satyendra Kumar[1].
[1]Kent State University, Kent, OH, USA, [2]Iowa State University, Ames, IA, USA, [3]Utrecht University, Utrecht, Netherlands.

Transport of fat in the bloodstream is mediated by lipoprotein particles. These hydrophobic particles are covered by a (phospho-)lipid monolayer and further stabilized by exchangeable apolipoproteins. Their amphipatic helix bundle is thought to unfold upon interaction with the (phospho)lipid monolayer. The dynamics of this interaction at lipid interfaces has not been directly shown experimentally. Here we report on the structure of monolayers formed by