water-soluble polymer, PEG, to the lamellar phase of DMPC or of several surfactants, induces a topological transformation to a vesicular phase. Such transition can be understood in terms of a modification of the elastic properties of the membranes. In this work we perform a dynamic light scattering (DLS) study of the effect of PEG on phospholipid and surfactant bilayers. The experimental results show that the addition of the polymer slows down the dynamics of the membranes. From fits to an available theory for the scattering of dilute membrane systems, we have calculated the bending elastic modulus of the bilayers. This modulus increases with increasing polymer concentration, thus confirming that the macromolecule modifies the elastic properties of the membranes.

Membrane Active Peptides II

1444-Pos

Effects of a Membrane-Active Amphibian Antimicrobial Peptide on the Bacterial Proteome

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Ribosomally synthesized antimicrobial peptides (AMPs) are conserved components of the innate immunity of all life forms and represent the most ancient and efficient weapon against microbial pathogens.

The emergence of multidrug-resistant microbes has urgently required the discovery of new antibiotics with a new mode of action, and AMPs represent promising candidates. Intense research focusing on AMPs is currently directed to the elucidation of their mode(s) of action.

Nevertheless, very little is known about their effects on intact bacteria.

Here we report on Esculentin 1-18 [Esc(1-18)], a linear peptide covering the first 18 N-terminal residues of the full length amphibian peptide esculentin-1b. Esc(1-18) retains the antimicrobial activity of esculentin-1b against a wide range of microorganims, with negligible effects on mammalian erythrocytes. To expand our knowledge on the molecular mechanism underlying the antimicrobial activity on Gram-negative bacteria, we investigated the effects of this peptide on *Escherichia coli*, by studying its: i) structure in membrane mimicking environment; ii) killing kinetic; iii) bactericidal activity in different media; iv) ability to permeate both artificial and bacterial membranes; v) capacity to synergize with conventional antibiotics; vi) effect on cell morphology and proteome by means of electron microscopy and proteomic techniques, respectively.

These studies have indicated that Esc(1-18) (i) kills *E. coli* via membrane-perturbation; (ii) elicits identical changes in the bacterium's protein expression pattern, at both lethal and sub-lethal concentrations; and (iii) preserves antibacterial activity under conditions closer to those encountered *in vivo*. This is in contrast with many host defence peptides that kill microorganisms by altering intracellular processes and lose activity in physiological solutions. Importantly, to the best of our knowledge, this is the first case showing the effects of an amphibian AMP on the protein expression profile of its bacterial target.

1445-Pos

A New Look at an Old Friend: Novel Insights Into Pore Formation by Alamethicin

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Alamethicin is a 20-amino acid long antibiotic peptide produced by the fungus *Trichoderma viride.* In the literature, alamethicin is the most commonly cited example of a barrel-stave model pore-forming peptide. In this model, amphipathic peptides form a long-lived transmembrane pore by aligning hydrophobic and hydrophilic residues with the lipid bilayer and aqueous pore respectively. It has been reported that voltage-independence of alamethicin pores relies on salt and peptide concentrations. We have developed a set of fluorescence-based assays for leakage, stable pore formation and lipid flip-flop using large unilamellar vesicles (LUVs) to help define the mechanism and potency of pore forming peptides. An additional pre-incubation assay differentiates dynamic pores from long-lived pores. When applied to alamethicin, our suite of assays show that, at 2.0 μM peptide, alamethicin forms voltage-independent pores in anionic or zwitterionic vesicles at peptide-to-lipid ratios as low as 1:2000. Less than 20 peptides per vesicle is sufficient to allow for complete vesicle permeabilization. Even after overnight incubation with vesicles, alamethicin promotes continuous lipid flip-flop, and is able to permeabilize multiple additions of new vesicles. Other pore forming peptides tested at this P:L ratio do not promote continuous flip-flop and do not exchange into new vesicles. We postulate that, unlike other pore-forming peptides, which mostly behave like classical barrel-stave pores, alamethicin is in a continuous dynamic equilibrium between transmembrane, interfacially bound and aqueous forms.

1446-Pos

Effect of L- to D-Peptide Isomerisation on the Activity of Antimicrobial Peptide Anoplin

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Isolated from the venom sac of solitary spider wasp, Anoplius samariensis, Anoplin is the smallest linear α-helical antimicrobial peptide found naturally up to date. It has broad spectrum activity against both Gram-positive and Gram-negative bacteria, and little hemolytic activity toward human erythrocytes (1,2). Previous studies showed that substitution of all amino acids in the peptide with D-isomers can increase the bioavailability and reduce peptide degradation without affecting the antimicrobial properties since the net charge and the hydrophobicity are retained (3). In the present work, two stereoisomers of Anoplin were studied using UV resonance Raman spectroscopy, Langmuir Blodgett, atomic force microscopy, calcein leakage assay and antimicrobial assay. UV resonance Raman data indicate that the two forms of the peptide adopt similar conformations in aqueous buffer and in membrane mimicking solutions. Monolayer isotherms show that D-Anoplin has a lightly greater area per molecule than L-Anoplin. Finally, membrane rupturing ability of both stereoisomers was found to depend strongly on membrane composition.

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1447-Pos

Biophysical Studies of Cecropin-Mellitin Antimicrobial Peptides with Improved Selectivity

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Antimicrobial peptides (AMPs) have received much attention as models for the development of antibiotics capable of meeting the challenge of drug-resistant infections. Our research has focused on a linear, 15-residue cecropin-mellitin (CM) hybrid peptide designated CM15. In these studies we compare the biological activities and membrane interactions of CM15 and lysine substituted derivates designed to have optimized amphipathicity upon membrane binding. Previous studies have shown that these lysine enriched peptides maintain the high antimicrobial activity of the parent peptide but have substantially decreased hemolytic effects (Sato and Feix, Antimicrob. Agents Chemother. 52, 4463, 2008). Using model membranes to understand the differences that govern peptide-membrane interaction, we have performed a series of biophysical experiments including circular dichroism (CD), fluorescence, and site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy. These experiments establish the structures of the membrane-bound peptides, determine the extent of membrane lysis, and allow determination of partition coefficients and depth of penetration. Our results show that differences in red cell hemolysis can be reconstructed using model membranes, and provide insights into the mechanism of membrane disruption. This work was supported by award number R01GM068829 from the National Institute Of General Medical Sciences.

1448-Pos

Structural Modifications to Convert Melittin from a Cytolytic Peptide to a Stable Cargo Linker

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Melittin is a 26 amino acid peptide that comprises more than half of the dry weight of the venom of the honeybee Apis Mellifera. In previous studies, we demonstrated that melittin stably bound to the lipid membrane of perfluorocarbon (PFC) nanoparticles and served as an active anti-cancer therapeutic agent *in vivo (Soman et al. J Clin Invest. 2009)*. Here, we report the structure modification that converts the mellittin to a cargo linker for **post-formulation** liposome customization. We introduced point mutations and truncations to define the lytic and membrane binding activities of melittin. For each of the mutations, lytic activity of the peptides was tested by the carboxyfluorescein fluorescence dequenching assay on carboxyfluorescein encapsulated liposomes. Among all six melittin mutations, the mutation, D1-7, with the first 7 amino acids removed (VLTTGLPALISWIKRKRQQ) dramatically reduces the melittin lytic activity. Using immobilized Giant Unilamellar Vesicles (GUV), we illustrated that at peptide:lipid ratio of 40:1, D1-7 bound to the GUV without pore forming, while at peptide:lipid ratio of 1:83, melittin already formed pores on the GUV