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

membrane. These results suggest that D1-7 eliminates the lytic activity but retains the strong lipid membrane binding. For further confirmation, we measured liposome sizes and zeta potentials with and without D1-7 loading. Consistently, D1-7 did not affect the size of the liposome, but shifted the zeta potential (or surface charge) of the liposome towards the positive voltage range, because D1-7 is a positively charged peptide. Among numerous existing nanosystems for drug delivery, liposomes are approved by FDA for anti-cancer and gene therapy. Accordingly, this linker and/or its refinements could enhance the therapeutic potential of approved liposomal drugs by enabling flexible incorporation and cargo multiplexing through post-formulation surface editing.

1449-Pos

Cationic, Helical Antimicrobial Peptoids with Biomimetic Antimicrobial Activity

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Increasingly prevalent resistance of pathogenic bacteria to conventional small-molecule antibiotic drugs is creating an urgent need for the discovery of new classes of antibiotics that are active against biofilms. Bacteria that are multidrug resistant (MDR) are of increasing concern for infectious disease. Current treatment of these infections that involve resistant organisms may require 6-12 months of antibiotic treatment, creating difficulties with compliance.

We are continuing to develop oligo-N-substituted glycine (peptoid) mimics of cationic, helical antimicrobial peptides (AMPs), and some of our recently acquired data indicate that peptoids could address the problem of growing resistance. Peptoids have been shown to have extremely broad-spectrum activity, and certain peptoids function well in the presence of serum proteins. Their biophysical mechanism of action makes it difficult for bacteria to evolve resistance to them. We have tested our most promising peptoids, peptides and commercial antibiotics in vitro against bacterial biofilms of a variety of important bacterial organisms. We show that certain peptoids can be as active as the preferred conventional antibiotics against bacterial infections, even at low micromolar doses. Small, structured biomimetic oligomers such as our antimicrobial peptoids may offer a new class of drugs that are useful in treating persistent bacterial infections.

1450-Pos

Investigation of a Sequence-Modified Antimicrobial Peptide

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Antimicrobial peptides serve as one of the first lines of defense in the immune systems of higher organisms. These peptides specifically target and neutralize infecting bacteria in the host organism while exhibiting little or no toxic effect on host cells. The peptide C18G is a highly cationic, amphiphilic peptide derived from the C-terminal sequence of the human protein platelet factor 4 (involved in blood coagulation and wound repair) exhibited antibacterial activity against both gram positive and gram negative bacteria. Using a modified C18G sequence that did not significantly affect antimicrobial efficacy (Tyr3 changed to Trp and all Lys changed to Arg (C18G Y3W K R)). The binding affinity was measured with fluorescence spectroscopy using the W in the peptide sequence as a probe of peptide environment. Small unilamellar lipid vesicles were used to investigate the binding affinity of the peptide to bilayers composed of variable amounts of DOPC, POPG, and POPE. DOPC and POPE have a zwitterionic head group, whereas POPG has an anionic charged head group. These studies showed binding affinity had a dramatic dependence on lipid composition. The effect of pH on peptide binding and behavior was also examined and, as expected, also impacted binding affinity. Quenching of the Trp fluorescence by acrylamide was performed to confirm that the Trp was located in the membrane. Likewise circular dichroism (CD) spectroscopy was used to determine the structure of the peptide upon interaction with the lipid vesicles. Additionally, in an assay monitoring membrane permeabilization of *E. coli* the C18G Y3W K R peptide was shown to permealize bacterial membranes in a concentration dependent manner.

1451-Pos

Structural Aspects of the Interaction of Nk-2 Derived Peptides with Cancer Cells

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Antimicrobial peptides have gained interest as potential anti-cancer agents, e.g. inhibition of tumour growth in human prostate xenografts was shown by host defense like lytic peptides (1). We showed by Annexin V binding that, in prostate tumour cells, negatively charged phosphatidylserine (PS) accumulates in the outer plasma membrane leaflet, which normally resides in the inner leaflet. Thus, surface exposure of PS may make these cells susceptible to killing by these cationic peptides.

The aim of this study is to develop short peptide sequences derived from NK-2, which was shown to have anti-tumour activity (2). NKCS (Cys of NK-2 exchanged by Ser) is composed of two α-helices connected with a hinge region. Initially we studied the interaction of the parent peptide and its N- and C-terminal part with membrane mimetic systems. Vesicle leakage experiments revealed that the N-terminal fragment exhibits similar affinity towards PS as NKCS. Thermodynamic experiments indicate that the N-terminal helix resembles the properties of NKCS. Furthermore, calorimetric studies revealed that NKCS and its fragments have no significant effect on the thermotropic behaviour of PC liposomes mimicking healthy mammalian cell membranes. Both circular dichroism and Monte-Carlo simulation using the bilayer parameters derived from our structural characterization of the lipid model systems showed that the selectivity for PS correlated with the alpha-helical content of the peptides. The C-terminal part was less structured showing lower affinity to PS containing membranes. Thus, the shorter N-terminal peptide can be used as a template for further optimization, as in vitro tests on a human prostate carcinoma cell line showed significant cell damage.

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1452-Pos

Antimicrobial Peptide Mimics as Potential Anticancer Agents:Interactions of Acyl-Lysine Oligomer C12K-7Alpha8 with Ganglioside/DPPC Mixtures

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Recently, antimicrobial peptides (AMPs) have emerged as a promising anticancer remedy. Negative charge of the bacterial membranes gives some measure for selectivity of cationic AMPs, since mammalian cell membranes are largely zwitterionic. Accumulating evidence indicates that lipid composition of the cancer cell membranes is different from a healthy cell, displaying net membrane surface negative charge. Understanding the nature of the negatively charged membrane domains could provide a new basis for anticancer therapy drug design using antimicrobial peptides or their synthetic mimics. Here, we examine the effect of membrane glycosylation, which is shown to be increased in cancer cells, on activity of AMP analogs. In this work we probe interactions of antimicrobial peptide mimic, based on acyl-lysine architecture (OAK), C_{12} K-7α_8, with Langmuir monolayers containing monosialoganglioside GM_3 and disialoganglioside GD_3. Langmuir isotherms and fluorescence microscopy imaging results of pure GM_3 and GD_3 monolayers indicate a single liquid-extended (LE) phase. Constant pressure insertion assays show significant insertion of C_{12} K-7α_8 in both GM_3 and GD_3 monolayers at 30mN/m. AMP analogue insertion was also observed for GM_3: DPPC (30:70) and GD_3: DPPC (30:70) mixed monolayers, however at smaller extent as expected. Synchrotron grazing Incidence X-Ray diffraction (GIXD) data show a disordered phase for GD_3 and a weak ordering for GM_3, which disappears immediately after introduction of the AMP. X-ray Reflectivity data indicate the thinning of the lipid layer upon peptide insertion.

1453-Pos

Cancer Cell Proliferation is Inhibited by Phlip Mediated Delivery of Membrane Impermeable Toxin Phalloidin

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We wish to use the pH-(Low)-Insertion-Peptide (pHLIP) to transport therapeutic agents to acidic tumors, with the ultimate goal of improving the treatment of cancer. pHLIP inserts into a lipid bilayer under slightly acidic conditions (pH 6-6.5), forming a transmembrane helix. We demonstrate here that pHLIP-mediated translocation of a cell-impermeable, polar toxin phalloidin can inhibit the proliferation of cancer cells. The delivery constructs, pHLIP-K(rho)C(aph) and pHLIP-C(aph), both carry the phalloidin toxin at the inserting C-terminus, via a disulfide linkage that could be cleaved in cells. The constructs differ in that a lipophilic rhodamine moiety is also attached to the inserting end, near the phalloidin cargo, in pHLIP-K(rho)C(aph). After a brief incubation with 2-4 μM of pHLIP-K(rho)C(aph) at pH 6.1-6.2 (for 1-3 h), proliferation of HeLa, JC, and M4A4 cancer cells were severely disrupted (> 90% inhibitions). Cells