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.

(1) Papo N. et al., Cancer Res. 66 (2006) 5371-8.
(2) Schröder-Borm H. et al., FEBS Lett. 579 (2005) 6128-61.

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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

treated with pHLIP-K(rho)C(aph) also showed signs of cytoskeletal immobilization, consistent with the knowledge that phalloidin binds to F-actin and stabilizes the filament against depolymerization. However, the antiproliferative effect was not observed with pHLIP-C(aph). The insertion behavior of both constructs were studied in POPC liposomes using Trp fluorescence: pHLIP-K(rho)C(aph) and pHLIP-C(aph) insert with the same apparent pK of 6.1-6.2, similar to that of pHLIP (without any cargo). However, kinetic experiments suggest that pHLIP-C(aph) inserts much slower than pHLIP-K(rho)C(aph), possibly accounting for its lack of antiproliferative effects in cell assays. In short, our results obtained with pHLIP-K(rho)C(aph) lay the foundation for the development of a new class of anti-tumor agents that would selectively enter and destroy cancer cells while not affecting normal cells. Such pHLIP-mediated delivery of otherwise cell-impermeable agents may enhance the efficacy of treatment, as well as significantly reducing the side effect.

1454-Pos

Membrane Superficial Charge Modification Affects Miotochondrial Permeabilization by Derivatives of the Polycationic Peptide Btm-P1

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Polycationic peptides demonstrate antimicrobial and anticancer properties. Earlier we designed, on the basis of the protoxin Cry11Bb, a 26-aa polycationic peptide BTM-P1, which demonstrated ionophoric and antimicrobial activities. It could be modified in the future to enhance anticancer action. In this work we found that the reverse peptide, BTM-RP1, has one order of magnitude lower capacity than BTM-P1 to permeabilize rat liver mitochondria. The activity of BTM-RP1 was increased by its modifications with tryptophane attached to its N-terminal (BTM-WRP1) or C-terminal (BTM-RP1W). The similar modifications of BTM-P1 peptide did not increase, or even decreased (BTM-P1W) the peptide activity. All these peptides, designed by us, were synthesized by Gen Script Company (USA) (>90% purity). When 10 μM cationic fluorescent probe safranin O, but not endogenous NAD(P)H fluorescence, was used as indicator of mitochondrial energization, the inner membrane potential markedly recovered after a decrease caused by each of 3 serial additions of 1 μM BTM-RP1. We also found that safranin O significantly decreased the rate of mitochondrial swelling induced by BTM-RP1 or by its tryptophane derivates. These data suggest that the superficial electrical charge of biomembranes, in addition to the trans-membrane potential, significantly affects the membrane permeabilization and selectivity in cell killing by polycationic peptides. We conclude that agents modifying superficial electrical charge of biological membranes could be used to influence the peptide cytotoxicity and selectivity. (Colciencias grant #111840820380 and the National University of Colombia grant #20101007930).

1455-Pos

Structure-Function Investigation of A Novel Dendrimeric and Lipidated Antimicrobial Peptide

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Antimicrobial peptides are usually polycationic with high affinity for bacterial membranes. Upon approaching the lipid bilayer, they tend to fold into an amphiphilic structure and bind to the membrane. In order to understand the detailed mode of action of such antimicrobial peptide inside the membrane, and to understand which properties of the peptide and/or lipids are important for selectivity, it is fundamental to examine the peptide structure and its association with lipid bilayers. In this work, first experiments were carried out to assess the thermodynamic and kinetic parameters of a promising novel antibiotic dendrimeric peptide interacting with lipid bilayers. With the goal of enhancing the antimicrobial activity of a particular sequence with the polyvalent framework of a dendrimer, two identical deca-peptides were assembled via a lysine-linker, carrying at the same time an octanoyl-lipid anchor. A highly active compound was obtained, but its structure and mode-of-action remain unexplored. The dendrimer and the linear deca-peptide were studied in parallel, to highlight the relevant properties and differences between dendrimeric structure and simple amino-acid sequence. Experiments were performed with different zwitterionic/negatively-charged lipids mixtures in order to assess the role of lipid surface charge. In particular, monolayer intercalation was investigated with microtensiometry. Fluorescence spectroscopy was applied to study thermodynamics and kinetics of the binding process. Circular dichroism, multidimensional liquid-state NMR, and solid-state NMR of oriented samples allowed to obtain first information on the 3D structure of the peptide both in the free and membrane-bound state. Transmission electron microscopy images showed the formation of highly intriguing aggregates with, to our knowledge, a previously unreported kind of branched three-dimensional morphology.

1456-Pos

Effects of Bacillus Lipopeptides on Lipid Membrane Structure and Dynamics

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Bacillus subtilis strain QST713 produces a unique combination of lipopeptides from the surfactin (SF), fengycin (FE) and iturin (IT) families. The fungicidal activity of this peptide mix is used by a biopesticide for crop protection and believed to be based on the permeabilization of target membranes by the peptides. To shed light on the activity, selectivity and synergisms of the peptides, we have studied their membrane binding and the subsequent effects on the structure and dynamics of the membrane. We measured the time-resolved fluorescence and fluorescence anisotropy of intrinsic tyrosine and hydrophobic dyes (e.g., DPH), time-resolved dipolar relaxation of Laurdan, interaction thermodynamics by ITC, and size and zeta potential of vesicles by DLS. The results are compared with the effects of synthetic surfactants and provide valuable information about the molecular background of the very unusual leakage and lysis behaviour of the lipopeptides.

1457-Pos

Rapid Binding and Transmembrane Diffusion of Pepducins in Phospholipid Bilayers

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Pepducins are GPCR-targeted lipopeptides designed to anchor in the cell membrane lipid bilayer and modulate the receptor/G protein signal transduction pathway via an allosteric mechanism. It is thus presumed that pepducins cross the plasma membrane by some mechanism, possibly passive diffusion. The goal of this research is to study the biophysical transport properties of pepducins in model membranes. We utilized fluorescent probes that measure the binding (fluorescein phosphatidylethanolamine - FPE) and diffusion (pH probe - pyranine) of charged ligands across the lipid bilayer of large unilamellar vesicles (LUV) comprised of egg-phosphatidylcholine. We tested pepducins with a palmitate or myristate linked to the N-terminal of the peptide sequence (KKSRALF). The GPCR target for these pepducins is the protease activator receptor 1 (PAR1). Addition of pepducins (0.16-5.0 mol%) to LUVs labeled in the outer leaflet with FPE or containing entrapped pyranine produced a fast (<2s) and dose-dependent increase in the fluorescence of both probes. The fast response of FPE, resulting from the insertion of positive charges (lysine and arginines residues) into the outer leaflet, demonstrated rapid partitioning into the membrane. The increase in pyranine fluorescence indicated alkalinization of the intravesicular compartment, probably due to protonation of the lysine residues. In order for this to be detected, the pepducin must cross the membrane. The peptide alone (not acylated) did not cause any change in the fluorescence of either FPE or pyranine. These data are consistent with favorable partitioning of pepducins into the membrane and rapid passive diffusion to the sites of their action at the cytosolic leaflet of the plasma membrane.

1458-Pos

Nanostructure Determines Antifungal Activity of De Novo Designed pH Dependent Histidine Containing Ultra-Short Lipopeptides

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Antimicrobial peptides are an essential part of the innate immune system of most living things and the understanding of the biophysical properties and the different mechanisms of action are crucial for the de-novo development of simple and effective analogs. More specifically, antimicrobial lipopeptides have been gaining increased attention because of the pressure for new antimicrobial agents against resistant pathogens. The addition of a lipophilic fatty acid has proven to be an effective method to increase the association of a peptide with the membrane, thus increasing the biological activity of certain peptide sequences. Previously, we reported that linear ultrashort cationic lipopeptides even as short as 4 amino acids have potent antimicrobial and antifungal properties. We described the minimum peptide length, and fatty acid length