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