of the bilayer differ significantly. This difference may alter the magnitude of the peptide's side chain implantation in the membrane and thus its activity. The solid-state NMR data collected on p1 and p3 will be used to create a high-definition structure using structure determination programs such as XPLOR.

445-Pos

Structural Studies of An Immune Modulating and Direct Antimicrobial Peptide

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The structure and function of the innate defence regulatory peptide 1018 was investigated. This peptide, whose sequence is distantly related to that of the 12 residue linear antimicrobial peptide Bac2A, a synthetic peptide derivative of the bovine cathelicidin Bactenecin, has both innate immune regulatory and direct antimicrobial activities. We present the solution state NMR structure of 1018 in DPC micelles, as well as its secondary structure in SDS and POPC/PG (1:1 molar ratio) from CD measurements. These structures reveal that 1018 can adopt a variety of folds, tailored to its different functions. The structural data is discussed in light of the ability of 1018 to induce cytokine and chemokine responses, to reduce the LPS-induced TNF-α response, and finally, to directly kill both Gram positive and Gram negative bacteria.

446-Pos

Determining the Charge State of Histidine Side Chains in Antimicrobial Piscidin By Nuclear Magnetic Resonance

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Piscidins constitute a family of three antimicrobial peptides discovered in the mast cells of hybrid striped bass. These peptides, which are highly cationic, contain several arginine and histidine residues. While piscidin 1 is the most antimicrobial and hemolytic isoform, piscidin 3, which has slightly lower antimicrobial activity, is significantly less hemolytic. One of the most striking differences between piscidin 1 and 3 is the substitution of glycine for the histidine at position 17 in piscidin 1.

As part of its mechanism of action, piscidin recognizes negatively charged microbial membranes. Therefore, studying the interactions of the piscidin with lipids can help us better understand the chemical basis of its antimicrobial and hemolytic effects. Because physiological pH is around 7.4, and the average pKa of histidine side chains is around 6.0, a detailed study of the histidine side chains in piscidin 1 and 3 is needed to discern the charge state of the peptides under physiological conditions. In this research, we used solution nuclear magnetic resonance to obtain the pKa of the histidine side chains of piscidin bound to sodium dodecyl sulfate micelles. Heteronuclear multiple quantum coherence experiments were performed on piscidin 1 and 3 containing ^{15}N-side chain labeled histidines. ^{15}N and ^{1}H chemical shifts were recorded as a function of pH to determine the titration curve of each histidine residue. The results will be discussed in the context of structure-function relationships in membrane-active peptides. The knowledge gained from these studies can help identify common principles that will facilitate the design of pharmaceuticals with broad-spectrum antibacterial activity, minimum induction of bacterial resistance, and low toxicity to mammalian cells.

447-Pos

Interaction of the Cationic Peptide Bactencin With DDPC/DMPG Phospholipid Mixtures At the Air-Water Interface

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In this work we show the results of the interaction of the cationic antimicrobial peptide bactenecin (Arg-Leu-Cys-Arg-Ile-Val-Val-Ile-Arg-Val-Cys-Arg) with DPPC/DMPG (X_{DPPC}= 0.5, X_{DMPG}= 0.5) mixtures using the Langmuir Through. The -A compression isotherms exhibit differences compared to those with DPPC alone, remaining the area per molecule, near 50 Å . The results obtained with atomic force microscopy indicate that mixed monolayers show a height near to 1.7 nm. Penetration of the dodecapeptide into the DPPC/DMPG mixtures at various surface pressures were investigated to determine the ability of this lipid monolayer to host the bactenecin. The higher penetration of peptide into phospholipids is attained when the monolayer is in the LC phase due to the control pressure applied (10, 15, 20 mN/m) and a greater interaction is allowed when DMPG is added in comparison with those monolayers of pure DPPC. The effect of bactenecin at the phospholipids' mixed monolayer was the shift of the LE phase at higher area per molecule. Circular dichroism of monolayers and multilayers of bactenicin/phospholipids were performed to investigate the peptide conformation.

448-Pos

LFampin Derived Antimicrobial Peptide: Biophysical Characterization and Biological Implications of Composition and Structure

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The innate immunity factor lactoferrin harbours two antimicrobial sequences situated in close proximity in the N1-domain, Lactoferricin (LFcin) and Lactoferrampin (LFampin). The more recently discovered LFampin by Jan Bolscher's group contains residues 268-284 from the N1 domain of Lactoferrin. Thereafter, a new family of antimicrobial peptides was obtained from LFampin by extension and/or truncation at the C- or N-terminal sides, keeping the essential characteristics, in order to unravel the main structural features responsible for antimicrobial action. These related synthetic peptides show broad-spectrum bactericidal activities against a range of Gram-positive and Gram-negative bacteria, as well as fungus. Bioactivity was tested towards pathogenic yeast *Candida albicans*and model bacteria strains.

The biophysical interaction with model membranes was studied by Differential Scanning Calorimetry (DSC), Isothermal Titration Calorimetry (ITC), Fluorescence Spectroscopy, Circular Dichroism, Zeta Potential and SAXD measurements.

Results will be presented for one of the peptides of this family, Lfampin 265-284, both regarding bioactivity and interaction with liposomes of DMPC, DMPG and DMPC:DMPG (3:1) as model membranes. Furthermore, the biophysical and biological implications of composition and structure will be discussed.

449-Pos

Roles of Lys and Arg in the Activity of Antimicrobial Peptides

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Antimicrobial peptides (AMPs) play a pivotal role in innate immunity. Most peptides kill microorganisms by permeabilizing cell membranes (e.g., magainin 2), although there are peptides targeting intracellular macromolecules, such as DNA (e.g., buforin 2). A common property of AMPs is polycationicity that enables the peptides to selectively interact with negatively charged bacterial surface. Some peptides (e.g., magainin 2) mainly contain Lys, and others (e.g., buforin 2) use Arg as a basic amino acid. To understand the roles of these amino acids in the activity of AMPs, we synthesized the magainin 2 and buforin 2 analogues.

The interaction with lipid bilayers were slightly enhanced by the K-to-R substitution because of a marginally larger hydrophobicity of Arg, and vise versa. In contrast to the membrane interaction, the substitutions significantly affected interaction with DNA. The Arg-containing peptides MGR and BF exhibited much stronger affinity for DNA than the Lys-containing counterparts. The antimicrobial activity of the membrane-acting magainin was not influenced by the K-to-R substitution, whereas that of the DNA-targeting buforin was lost by the R-to-K substitution.

450-Pos

Characterization of Indolicidin-Membrane Interactions By Simultaneous Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy-Atomic Force Microscopy

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A detailed understanding of how antimicrobial peptides interact with bacterial membranes is a key step towards the effective design of novel antibiotics to treat infection. These interactions may include membrane-induced conformational changes to the peptide, membrane disordering, as well as peptide aggregation. To understand the effect of both membrane composition and peptide sequence on these phenomena, we applied simultaneous attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR)-atomic force (AFM) microscopy to directly visualize and characterize the interactions of the model antimicrobial peptide, indolicidin, with a series of supported planar lipid bilayers. This approach allows us to directly interrogate how peptide association, aggregation, and insertion alter the structure of the bilayer. It also allows us to directly assess changes to the secondary structure of the peptide as a consequence of both specific peptide-membrane interactions as well as peptide-peptide interactions. Simultaneously acquired AFM images provide direct confirmation of the effect of the peptide on membrane integrity, evidence of domain targeting, as well as the kinetics and structure of putative peptide