monounsaturated acyl chain. Except for the third series, peptide activity correlated with the first moment of the lateral pressure profile, which is a function of lipid acyl chain structure. In vesicles composed of asymmetric phosphatidylcholines, peptide binding and dye efflux are enhanced compared to symmetric, unsaturated lipids with similar pressure profiles. We attribute this to the entropically more favorable interaction of delta-lysin with partially saturated phospholipids. We find that lipid acyl chain structure has a major impact on the activity of delta-lysin and is likely to be an important factor contributing to the target specificity of amphipathic peptides.

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Magainin 2 Revisited: a Test of the Quantitative Model for All-or-None Permeabilization of Phospholipid Vesicles

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The all-or-none kinetic model that we recently proposed for the antimicrobial peptide cecropin A is tested here for magainin 2. In mixtures of phosphatidylcholine (PC) /phosphatidylglycerol (PG) 50:50 and 70:30, release of contents from lipid vesicles occurs in an all-or-none fashion and the differences between PC/PG 50:50 and 70:30 can be ascribed mainly to differences in binding, which was determined independently and is about 20 times better for PC/PG 50:50 than to 70:30. Only one variable parameter, beta, corresponding to the ratio of the rates of pore opening to pore closing, is used to fit dye release kinetics from these two mixtures, for several peptide/lipid ratios ranging from 1:25 to 1:200. However, unlike for cecropin A where it stays almost constant, beta increases 5 times as the PG content of the vesicles increases from 30 to 50 percent. Thus magainin 2 is more sensitive to anionic lipid content than cecropin A. But overall, magainin follows the same all-or-none kinetic model as cecropin A in these lipid mixtures, with slightly different parameter values. When the PG content is reduced to 20 mole percent, dye release becomes very low; the mechanism appears to change, and is consistent with a graded kinetic model. We suggest that the peptide may be inducing formation of PG domains. In either mechanism, no peptide oligomerization occurs and magainin catalyzes dye release in proportion to its concentration on the membrane in a peptide state that we call a pore. We envision this structure as a chaotic or stochastic type of pore, involving both lipids and peptides, not a well-defined, peptide-lined channel.

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Influence Of The Bilayer Composition On The Membrane-Disruption Effect Of Polybia-MP1, A Mastoparan Peptide With Antimicrobial And Leukemic Cell Selectivity

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Unlike other mastoparans, Polybia-MP1 (IDWKKLLDAAKQIL), from the venom *Polybia paulista* (wasp), is highly selective for bacterial cells. By flow cytometry, we also found out this selective behavior: Polybia-MP1 promoted a decrease of 60 % cell viability at 25 μM in Jurkat (leukemic) cells, while it was not altered in primary human lymphocytes. The mechanism of selectivity was studied in the interaction with different bilayers. Ion channel-like activity was detected at 0.12 μM peptide concentration with anionic lipid membranes of azolectin, showing conductance in the range of 250 pS. On zwitterionic diphytanoylphosphatidylcholine-(DPhPC) it required 0.18 μM for the same conductance level. Further experiments with DPhPC bilayers containing 30% phophatidylserine or cardiolipin required higher peptide concentration to induce single channel events at slightly lower conductance levels. However, the presence of 20 mol% cholesterol in the mixture significantly reduced the ion channel-like activity, dropped the average conductance to around 120 pS and required 0.30 μM. On vesicles the activity of Polybia-MP1 also shows greater rate of leakage on the anionic over the zwitterionic, impaired by the presence of cholesterol; the lytic activity is characterized by a threshold peptide to lipid molar ratio that depends on the phospholipid composition. Preliminary results of changes in DPH anisotropy and acrylamide quenching of Trp fluorescence show a slight decrease in the anisotropy, and a significant quenching of the Trp fluorescence, indicating small influence on the lipid packing associated to preferential interaction with the lipid head group region. Results suggest that the selectivity of Polybia-MP1 is a consequence of a shallow interaction with zwitterionic bilayers, favored by the presence and position of negatively charged Asp residues, which is not possible for other mastoparan peptides.
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Effect of Antimicrobial Peptides from Australian Tree Frogs on Anionic Phospholipid Membranes

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Skin secretions of Australian tree frogs contain antimicrobial peptides that form part of the host defence mechanism against bacterial infection. The mode of action of these antibiotics is thought to be lysis of infectious organisms via cell membrane disruption, on the basis of vesicle-encapsulated dye leakage data [Ambroggio et al., (2005) Biophys. J. 89, 1874-1881]. A detailed understanding of the interaction of these peptides with bacterial membranes at a molecular level, however, is critical to their development as antibacterial therapeutics. We focus on four of these peptides, aurein 1.2, citropin 1.1, maculatin 1.1 and caerin 1.1, which exist as random coil in aqueous solution, but have α-helical secondary structure in membrane mimetic environments. In our earlier solid-state NMR studies, only neutral bilayers of the zwitterionic phospholipid dimyristoylphosphatidylcholine (DMPC) were used. Deuterated DMPC (d54-DMPC) was used to probe the effect of the peptides on the order of the lipid acyl chains and dynamics of the phospholipid head groups by deuterium and 31P NMR, respectively. We demonstrate several important differences when anionic phospholipid is included in model membranes. Peptide-membrane interactions were characterised using surface plasmon resonance (SPR) spectroscopy and solid-state NMR spectroscopy. Changes in phospholipid motions and membrane binding information provided additional insight into the action of these antimicrobial peptides. While this set of peptides have significant C- and N-terminal sequence homology, they vary in their mode of membrane interaction. The longer peptides caerin and maculatin exhibited properties that were consistent with transmembrane insertion while citropin and aurein demonstrated membrane disruptive mechanisms. Moreover, aurein was unique with greater perturbation of neutral versus anionic membranes. The results are consistent with a surface interaction for aurein 1.2 and pore formation rather than membrane lysis by the longer peptides.

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The Alignment of Membrane-Active Peptides Depends on the Lipid Phase State as Viewed by solid state 19F-NMR

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Amphipathic membrane-active peptides (antimicrobial, hemolytic, cell-penetrating, fusogenic, etc.) achieve their functions by distinct interaction with lipid bilayers. Some typical structural modes are described in terms of models like the ''barrel stave'', ''toroidal pore'', ''carpet'' etc. These models are related to the alignment states of the peptides in the lipid bilayers (surface bound ''S-state'', inserted ''I-state'' or tilted ''T-state''), which can be readily characterized by solid state NMR. When determining such alignment, factors like peptide/lipid ratio, charge of the bilayer surface, thickness of the bilayer core, presence of cholesterol, and humidity are typically investigated. Yet, the lipid phase state as an explicit variable parameter has not received much attention so far. Here, we demonstrate that a change in the lipid phase can directly trigger the re-alignment of many peptides. Several representative examples are illustrated here: PGLa, PGLa/Magainin, gramicidin S, SAP and alamethicin. In macroscopically oriented DMPC bilayers, using highly-sensitive 19F-NMR we have monitored the changes between known alignment states of these peptides as a function of temperature, covering both the gel and liquid-crystalline states of DMPC. We show that for all peptides studied the alignment in the gel-state differs from the one in the liquid-crystalline bilayers and can be reversibly changed by passing through the lipid phase transition temperature. The relevance of these finding for the phase state of native biological membranes and interactions of membrane-active peptides with them will be discussed.