1499-Pos Board B229

Redistribution of Cholesterol in Model Lipid Membranes in Response to Alamethicin

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Cell membranes are complex mixtures of lipids, proteins and other molecules that serve as active, semipermeable barriers between cells and their internal organelles and the surrounding medium. Cell membrane molecular and macromolecular compositions are tightly regulated to ensure proper function. Cholesterol is a key component in mammalian cellular membranes, where it serves to maintain membrane fluidity and permeability. Here, the interaction of alamethicin, a 20 amino acid residue peptide that creates transmembrane pores in lipid bilayer membranes in a concentration-dependent manner, with cholesterol (Chol) containing dimyristoyl phosphatidylcholine (DMPC) membranes. Small-angle neutron scattering (SANS) data demonstrate that a low concentration of alamethicin (lipid to peptide ratio of 200:1) disrupts the lateral inhomogeneity seen in peptide-free DMPC:Chol vesicles, which is a coexistence of different phases. The resulting laterally heterogeneous bilayers are thinner than the peptide-free Lo phase, and possess a stronger asymmetry in the Chol content of the inner and outer bilayer leaflets. The results point to an alternative to the well-understood cytotoxic membrane permeabilization mechanism of action, specifically that membrane-active peptides are capable of disrupting lipid rafts and other functional structures in cell membranes.

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Screening the Insertion of Families of Bioactive Microbial Metabolites into Tethered Bilayer Lipid Membranes (TBLMS)

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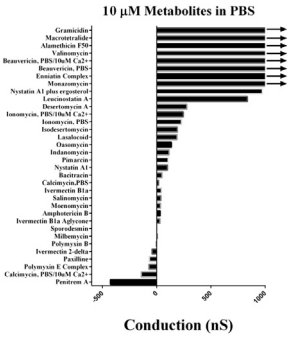
While it is known that many microbial metabolites can permeabilize phospholipid membranes, it is not easy to find quantitative comparisons across large numbers of such metabolites. Such comparisons are of use in screening likely candidates for ongoing research into the development of new and improved antibiotics. We have studied the effects of 30 microbial metabolites on the electrical conductivity of a tethered bilayer lipid membrane comprising two standard phospholipid components. The technique allows rapid quantification of the activity of these compounds. As well as the electrical conductivity, we have analyzed the changes in membrane *capacitance* by the insertion of metabolites which reports on the change of membrane thickness, as well as the introduction of water molecules into the membrane. Using a standard 10 μM concentration for each of the 30 metabolites, we rank them according to their impact on membrane conduction in PBS solution (Fig 1). We also demonstrate that by determining the change in conduction (ΔG) against the change in capacitance (ΔC) caused by membrane insertion it is possible to determine the complexation properties of the metabolites as they insert into the membrane.



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NMR Characterization of Spider Venom Neurotoxin Structure and Interactions with Lipid Bilayers

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Spider venom is comprised of a diverse range of molecules including salts, small organics, acylpolyamines, peptides and proteins. The peptides are by far the largest and most diverse range of biomolecules found in spider venoms. These peptides have considerable pharmacological activity. Peptides isolated from the Chilean Rose tarantula, Grammostola Rosea (G. Rosea), have been shown to target and modulate voltage activated K^+ channels, Na^+ channels, Ca^{2+} channels and mechanosensitive ion channels (MSCs). These peptides are cysteine-rich and fold in an inhibitor cysteine knot (ICK) motif. Here, we present the molecular structure of one G. Rosea neurotoxin, GsAF2, determined by solution-state nuclear magnetic resonance spectroscopy (NMR). In addition, neurotoxin interactions with lipid bilayers for GsAF2 and VsTx1 are probed with a suite of solid-state NMR techniques including 31P static, 31P magic angle spinning and spin-lattice (T1) relaxation measurements. Finally, cryo-transmission electron microscopy (TEM) experiments illustrate that the neurotoxins influence lipid bilayer morphology with the formation of a toxin-lipid nematic phase. These studies elucidate differences in ICK neurotoxin three-dimensional (3D) structure and their interactions with the lipid bilayer.

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Structure Activity Relationship for a Synergistic Pair of Antimicrobial Peptides from the Magainin Family

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The antimicrobial peptides Magainin 2 (Mag2) and PGLa are both found in the skin of the African frog *Xenopus laevis*. They show high antimicrobial activity against bacteria, fungi, and cancer cells.[1] Both peptides form amphipathic α-helices upon binding to a lipid bilayer. Previous studies revealed that mixtures of Mag2 and PGLa show synergistic effects,[2] and the formation of stable 1:1 heterodimeric peptide pores has been proposed as a possible mechanism.

The membrane alignment of each of these two peptides *per se* has been determined with high accuracy using solid state ^{2}H-, ^{15}N-, and ^{19}F-NMR spectroscopy. Using solid state ^{2}H-NMR, we have previously characterized the orientation of PGLa on its own and in the presence of an equimolar amount of Mag2, and found an inserted transmembrane orientation of PGLa in DMPC/DMPG only in the presence of Mag2.[3] On the other hand, Mag2 always stays flat on the surface of the lipid bilayer, both on its own and in the presence of PGLa.

The aim of the present study was to determine the detailed local interaction between the two peptides in the membrane-bound state, taking hydrophobic and charge interactions into account. Several mutations were introduced at potentially crucial positions of either peptide and investigated by ^{15}N-NMR and biological assays. It was found that synergy is correlated with the insertion of PGLa, and important residues for synergy could thus be identified.

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Unveiling the Membrane-Binding Properties of N-Terminal and C-Terminal Regions of G Protein-Coupled Receptor Kinase 5 by Combined Optical Spectroscopies

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G protein-coupled receptor kinase 5 (GRK5) is thought to associate with membranes in part via N and C-terminal segments that are typically disordered based on available high resolution crystal structures. Herein we investigate molecular interactions of these regions with model cell membrane using combined sum frequency generation (SFG) vibrational spectroscopy and attenuated total reflectance (ATR)-FTIR spectroscopy. It was found that both regions associate with POPC lipid bilayers but adopt different conformations: GRK5-2-31 was in random coil while GRK5-546-565 was partially helical. When the subphase for the GRK5-2-31 peptide was changed to 40% TFE/60% 10 mM phosphate pH=7.4 buffer, a large change in the SFG amide I signal indicated that GRK5-2-31 became partially helical. By inspecting the membrane behavior of two different segments of GRK5-2-31, namely, GRK5-2-24 and GRK5-25-31, we found that residues 25-31 are responsible for membrane binding, whereas the helical character is imparted by residues 2-24. With SFG, we deduced that the orientation angle of the helical segment of GRK5-2-31 is $46 \pm 1°$ relative to the surface normal in 40% TFE/60% 10 mM phosphate pH=7.4 buffer and $78 \pm 11°$ in 40% TFE/60% pH=7.4 PBS buffer. We also investigated the effect of PIP2 in the model membrane and concluded that the POPC:PIP2 (9:1) lipid bilayer did not change the behavior of either peptide compared to a pure POPC lipid bilayer. With ATR-FTIR, we also found that Ca^{2+}•calmodulin is able to extract both peptides from the POPC lipid bilayer, consistent with the role of this protein in disrupting GRK5 interactions with the plasma membrane in cells.