been previously shown to simulate the phase properties of the lipid components in lung surfactant bilayers and monolayers. Presence of native palmitoylated SP-C reduced the size of lo domains in the DPPC/DOPC/cholesterol membrane model as detected by Förster Resonance Energy Transfer (FRET). Interestingly, very similar effects on the lo/ld equilibrium could be observed in the presence of a recombinant variant of SP-C, in which the two palmitoylcysteines of the native protein had been replaced by phenylalanines. It has been suggested that phenylalanines can act as functional mimics of palmitoylated cysteines in SP-C from some animal species. We therefore propose that the effects of SP-C on domain size could be related to selective interactions of this protein with liquid-ordered membrane regions and that this could be important for SP-C-promoted stabilization of lung surfactant films *in vivo*.

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Molecular Dynamics Simulations of Model Lung Surfactant Monolayers and Surfactant Protein B Fragment

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Surfactant Protein B (SP-B) plays an essential role in the proper functioning of lung surfactant. However, the details of how SP-B interacts with lung surfactant lipids to support lung function are poorly understood. The interactions between an SP-B based peptide and lung surfactant lipid monolayers are investigated using molecular dynamics simulations. Mini-B, a peptide of 34 amino acid residues consisting of the N-terminal and C-terminal alpha helices of full-length SP-B, achieves a similar level of function to full length SP-B in rodent models of respiratory distress. The monolayers probed included pure DPPC, pure POPG, and a mix of 7:3 DPPC:POPG. First the most stable configuration of the peptide-monolayer system is sought by allowing the system to evolve through time from different starting configurations and peptide orientations. It is then determined what effect the counter-ion concentration has on the screening of the electrostatic interaction between the negatively charged headgroups of POPG and the positively charged residues of the peptide. Finally, by placing a bilayer of lipids adjacent to the monolayer, in resemblance to the coexisting lipid reservoirs, the influence Mini-B has on the interaction between the monolayer and bilayer is demonstrated.

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Interactions of SP-B Based Peptide with Lipid and Protein Components of Lung Surfactant

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Lung surfactant (LS) is a mixture of lipids and proteins that reduces the surface tension at the alveolar air-water interface and thus prevents lung collapse and enables normal breathing. Surfactant Protein B (SP-B) is an essential component of LS and is absolutely necessary for survival. SP-B is thought to function by facilitating large-scale rearrangements of lipids and stabilizing the structures at various stages of the breathing cycle. However, neither the structural basis for this ability nor the physiological ramifications of lipid rearrangements are yet understood, in part because a high-resolution structure of SP-B is not yet available. Mini-B is a peptide fragment of SP-B that has been shown in *in vitro* and *in vivo* studies to retain similar activity to the full-length protein. Previously, we determined the structure of Mini-B, first in organic solvent hexafluoroisopropanol (HFIP) and then in detergent micelles composed of sodiumdodecylsulfate (SDS) using solution NMR. In our present work, we have studied the interactions of Mini-B with dodecylphosphocholine (DPC) and SDS micelles. DPC and SDS micelles provide an interfacial environment, with lipid headgroups corresponding to the headgroups of the most abundant lipids in LS, phosphatidylcholine (PC) and phosphatidylglycerol (PG) and thus solution NMR studies of interactions between Mini-B and these micelles can provide insight into LS protein-lipid interactions. We have also investigated the interactions of Mini-B with the most abundant surfactant protein SP-A under similar conditions. These studies further the understanding of the mechanisms of SP-B interactions with other surfactant components in native lung environment.

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Characterization of Transmembrane Peptide-Anchored Lactoferricin in Mixed Lipids

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To investigate the effects of a cationic juxtamembrane sequence on a hydrophobic transmembrane domain, model peptides have been designed with the lactoferricin sequence RRWQWR (LfB) anchored to an α-helical transmembrane peptide (RRWQWR-(spacer)-$(LA)_7$KKA). Spacers to date have included the helix-breaking -GPGG- and the helix-continuing -AA- sequences. The transmembrane domain contains a hydrophobic $(Leu\text{-}Ala)_7$ helical sequence that spans the membrane with two lysine anchors at the C-terminus. Selected alanines, deuterated on the C_α and C_β carbons, were incorporated in the sequences and used for solid-state NMR spectroscopy. Circular dichroism spectra reveal that the presence of the -AA- spacer correlates with a higher helix content in both DMPC and DMPC:DMPG (3:1) bilayer membranes. Solid state ^2H NMR spectra of macroscopically aligned lipid:peptide samples on glass plates reveal in all cases signals from the $C_\beta D_3$ groups and in some cases signals from the individual C_α deuterons. For example, in DMPC and DMPC:DMPG, the $C_\beta D_3$ groups for the peptide with the -GGPG-spacer give very similar quadrupolar splittings, in the range of 7-10 kHz. Interestingly, in DMPC the C_α deuterons, which are often not observed in similar transmembrane peptides, can also be seen for both labeled alanines, at 48 kHz and 70 kHz. In DMPC:DMPG a single $C_\alpha D$ quadrupolar splitting is resolved at 65 kHz. The presence or absence of selected $C_\alpha D$ resonances in NMR spectra of RRWQWR-(spacer)-$(LA)_7$KKA as well as WALP19, each with or without proline, will be considered in oriented samples of different bilayer lipid compositions.

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Interaction of the Protein Retinitis Pigmentosa 2 (RP2) with Langmuir Phospholipid Monolayers

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A severe form of retinitis pigmentosa is linked to mutations of the 350 residues protein RP2 (retinitis pigmentosa 2). This protein contains a α/β C-terminal domain, a highly hydrophobic β-helix and two acylation sites at the N-terminal. It localizes predominantly to the membrane. However, the parameters responsible for the modulation of RP2 binding to membranes are still largely unknown. The objectives of this research work were to characterize the membrane binding properties of RP2 using Langmuir monolayers. The complete sequence of RP2 was expressed and high purity was achieved. RP2 was injected into the subphase underneath phospholipid monolayers bearing different fatty acyl chains (length and unsaturation) and polar headgroups. RP2 binding was monitored by surface pressure measurements. The injection of RP2 underneath phospholipid monolayers led to an increase in surface pressure which indicates its membrane binding. The surface pressure data demonstrate that the adsorption kinetics of RP2 is independent of pH but is strongly affected by the ionic strength of the subphase as well as by the type of phospholipid fatty acyl chain (length and unsaturation) and headgroup. For example, on the basis of its maximum insertion pressure, RP2 shows a preferential binding onto saturated phospholipid monolayers which is consistent with its postulated localization to rafts. This interaction has been further studied by infrared sprectoscopy. In solution, the amide I band is centered at 1630 cm^{-1}, indicating the presence of the β-helix. In contrast, when injected into the subphase in the absence and presence of a phospholipid monolayer, the amide I band is shifted to longer wavenumbers with components at 1640 and 1655 cm^{-1}. These data thus suggest that RP2 has a preferential orientation in monolayers where the α/β C-terminal domain is oriented towards the monolayer.

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Influence of the Lipidation Motif on the Partitioning and Association of N-Ras in Model Membrane Subdomains

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In a combined chemical biological and biophysical approach using time-lapse tapping-mode atomic force microscopy, we studied the partitioning of differently lipidated N-Ras proteins with various membrane-recognition motifs into lipid domains of canonical model raft mixtures. The results provide direct evidence that partitioning of N-Ras occurs preferentially into liquid-disordered lipid domains, independent of the lipid anchor system. N-Ras proteins bearing at least one farnesyl group have a comparable membrane partitioning behavior and show diffusion of the protein into the liquid-disordered/liquid-ordered phase boundary region, thus leading to a decrease of the unfavorable line tension between domains. In addition, except for the monofarnesylated N-Ras, strong intermolecular interactions foster self-association and formation of nanoclusters at the domain boundaries and may serve as an important vehicle