of the monolayer film. Alterations in these properties in the absence of surface active lung surfactant proteins were also observed indicating that interactions between the monolayer at the interface, the ''multilayer reservoir'' and the subphase are essential for the proper functioning of the lung surfactant.

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The effect of proteins and cholesterol on viscoelastic properties and morphologies of Lung Surfactant system

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One of the essential features of healthy lung surfactant (LS) is to reduce the surface tension as well as increases the surface viscosity at the alveolus air-water interface to prevent the collapse. Currently, there is no simple theory explaining physicochemical properties of a monolayer with respect to surface tension or surface viscosity in the dynamic process of breathing. Monitoring the viscoelastic properties of interfacial films allows to predict how such a property depends on lipid and protein composition, packing properties and other variables at different states of the breathing process. Cholesterol and proteins are believed to have drastic effects on lung surfactant system by changing surface shear viscosities but systematic studies regarding this have not been done yet and the compositional effects remained still unkown. Here we focus on the influence of (1) cholesterol and (2) LS proteins such as SP-B_{Mini} and SP-C_{ff} on the surface viscosity and surface tension of pure DPPC monolayer as well as the lung surfactant replacement Survanta.

In order to study the compositional effects on the surface viscosity and surface tension, we have used custom-made Langmuir trough as a viscometer. Our viscometer consists of a Langmuir trough equipped with Helmholz coils which generate a controlled magnetic force to move a magnetic needle floating on the monolayer. The viscometer enables to examine the surface viscosity and surface tension as well as morphological changes in a LS monolayer as varying the amount of cholesterol and LS proteins. Atomic force microscopy has been also used to further investigate the morphological changes of a LS monolayer in nanometre regime after prepared by Langmuir Blodgett technique.

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Interaction of Lung Surfactant Protein A with Phosphatidylcholine Vesicles

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Surfactant protein A (SP-A) , a member of the collectin family found in the lung, binds to dipalmitoylphosphatidylcholine (DPPC), and plays roles in the formation of tubular myelin and the regulation of uptake and secretion of surfactant lipids by alveolar type II cells. The calcium dependent binding of SP-A to both small (SUV) and large (LUV) unilamellar vesicles of phosphatidylcholines (PC) has a dramatic effect on the PC dynamics as measured by ^{1}H linewidths of the acyl chains. For fluid bilayers (dipalmitoyl-PC at 42°C, dimyristoyl-PC and 1-palmitoyl-2-oleoyl-PC at 37°C), ω-CH_3 as well as bulk (CH_2)n and $N(CH_3)_3$ resonances are broadened with the addition of SP-A above a molar ratio of 0.02 SP-A/PC. This binding does not cause SUV or LUV fusion as monitored by negative staining TEM. However, the SP-A binding to PC vesicles exhibits two phases, one where the chain resonances are constrained and a second, requiring increased Ca^{2+} or the presence of 10 mol% cholesterol, characterized by a new downfield $(CH_2)_n$ resonance. The addition of EDTA can partially reverse the second phase of SP-A binding. The changes in PC acyl chain behavior provide insight into how SP-A interacts with multilamellar bodies and how it may aid in insertion of dipalmitoyl-PC into the air-water interface of the lung.

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Studying the Effects of Protein and Lipid Composition on Lung Surfactant Adsorption Through Confocal Microscopy

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Lung surfactant (LS) is a mixture of lipids and proteins that lines the air-water interface of the alveolar walls. It modulates the surface tension in the lungs which greatly reduces the mechanical work of breathing and also prevents the collapse of the alveoli upon expiration. Although lipids are the major constituent of LS, the hydrophobic surfactant proteins SP-B and SP-C play an integral role in proper adsorption of LS to the air-water interface. Understanding the role of these proteins will allow for better design of exogenous LS therapies for the treatment of respiratory distress syndrome. Confocal microscopy's abilities to optically section a sample and simultaneously image multiple dyes provide an ideal tool to study LS adsorption *in vitro*. Combining three-dimensional, multi-component imaging with surface tension measurements allows us to determine whether a particular surfactant mixture can successfully transition from bilayer aggregates in the bulk to a functional monolayer on the interface. SP-B and SP-C are believed to play an integral role in both the transport of aggregates to the interface and the unfolding of surfactant bilayers into a monolayer. Confocal microscopy has allowed us to study the importance of lipid and protein composition on the transport and unfolding of LS.

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Membrane Partitioning of Mechanosensitive Channel Inhibitor GsMTx4: Characterization by Depth-Dependent Fluorescence Quenching and Molecular Dynamics Simulations

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Recently we have demonstrated that membrane partitioning is a property of some (but not all) Inhibitor Cysteine Knot ion channel blockers [Biophysical J. 2007, 93:L20]. GsMTx4 is the only one among the studied blockers that interacts with anionic and zwitterionic lipids with nearly equal affinity. To gain insight into the determinants of its bilayer interactions we have examined several of its mutants and found to our surprise that none of the mutations had significant effects on membrane partitioning. Another surprising feature of GsMTx4 is the almost complete absence of changes in intrinsic fluorescence during membrane insertion. Penetration of GsMTx4 into lipid bilayer was determined using Distribution Analysis of the depth-dependent fluorescence quenching [Biophysical J. 1999, 76:946]. This analysis indicates that GsMTx4 and its W6A mutant penetrate into the bilayer deeper than Melittin. To interpret the fluorescence data and to elucidate peptide-lipid interactions involved in binding of GsMTx4 to PC membranes we performed MD simulations, which showed that anomalous fluorescence behavior of GsMTx4 on membrane partitioning is caused by water penetration into the lipid-peptide interface. The MD simulations also demonstrated high lipid perturbation and preferential interactions of cationic side chains of the peptide with lipid phosphate groups. Acknowledgments: I am grateful to F.Sachs for the gift of GsMTx4, to D.J.Tobias for performing MD simulations and to A.S.Ladokhin for helpful discussions. This research was supported by NIH Grant GM-069783 (A.S.Ladokhin) and KUMC Biomedical Research Training Program Fellowship (Y.O.Posokhov).

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Phase Transition Behaviors And Interactions And Adhesion Of Myelin Lipid Membranes Modulated By Myelin Basic Protein

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Myelin is a stacked membrane structure that allows for fast, efficient conduction of nerve impulses. It has 8 kinds of lipid molecules on two alternating bilayers and proteins such as myelin basic protein (MBP) which has an important role in maintaining myelin structure. The compact bilayer organization of healthy myelin is believed to require a well-defined range of lipid and protein composition, and lipid-protein interaction. Even though we know that multiple sclerosis (MS) is a morphological transformation involving loss of adhesion between myelin lamellae and sometimes formation of myelin vesicle, its mechanism and causes for demyelination are still under investigation.

We have used fluorescence microscopy, Langmuir isotherm, and Langmuir-Blodgett (LB) techniques to investigate how lipid composition of myelin lipid system affects the phase transition behaviors of myelin monolayers and bilayers depending on lateral pressure, temperature, and pH conditions. Model membranes with the composition of the cytoplasmic side of experimental allergic encephalomyelitis (EAE) myelin were also constructed on mica surfaces by LB deposition and the forces between the surfaces measured using the Surface Forces Apparatus (SFA) after exposure to various solution concentrations of MBP.

Our findings clearly demonstrate EAE monolayer remains phase-separated under physiological conditions. If the myelin sheath were to form two phases in vivo there are a variety of effects that could result. The line tension between two segregated domains and a local repulsive force could cause the membrane to bulge leading to vesiculation of the membrane. Force-distance measurements between supported myelin bilayers mimicking the cytoplasmic surface of myelin at various surface coverages of MBP indicate that maximum adhesion and minimum cytoplasmic spacing occurs when each