have observed that KL4 containing monolayers demonstrate an increased tolerance to repeated compression and expansion due to a softening in folding collapse behavior caused by direct interactions with POPG. This change in folding dynamics leads to increased monolayer reversibility due to almost complete reincorporation of folds upon expansion. We will discuss the potential role of KL4 in lowering the resistance to in-plane shear in POPG containing monolayers in the context of the overall importance of collapse mode in establishing robust and reversible synthetic model lung surfactant.

2857-Plat
Exploring Supramolecular Aspects of the Effect of Sphingomyelinase D On Sphingomyelin-Containing Membranes

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Lipid-modifying enzymes play a vital role in the regulation of lipids as mediators of cell function. At the same time, the activity of these enzymes is highly affected by the lipid membrane structure. These processes at lipid membranes can be observed in situ through the application of different biophysical techniques. Thus, we are investigating a spider venom enzyme termed sphingomyelinase D (SMD). SMD hydrolyses sphingomyelin (SM) into ceramide-1-phosphate (Cer-1-P). While SM is an integral constituent of many cell membranes, Cer-1-P occurs in very low concentrations and is suggested to be a novel lipid second messenger. At present, the physiologically relevant mechanism following Cer-1-P formation by SMD is incompletely understood, but possibly related to the modulation of membrane properties.

Our results show a strong dependency of SMD activity on the phase state of the substrate. SMD is two orders of magnitude more active towards fluid-than gel-phase liposomes. The presence of cholesterol events out this difference in activity at an intermediate level. The effect of SMD on fluid-phase giant unilamellar vesicles (GUVs) is observed by confocal fluorescence microscopy. GUVs composed of lauroyl-SM show a macroscopic domain formation and/or shrinking and buckling accompanied by the multiple formation of membrane tubes. GUVs composed of egg-SM display a beveling of the membrane and the formation of caps (outside curvature) approx. three days after the addition of SMD. Which membrane morphology evolves is likely a question of enzyme kinetics vs. the dynamics of lipid reorganization. GUVs of raft-like mixtures exhibit a single homogenous phase after the addition of SMD. The consequences of SMD activity and Cer-1-P formation on cellular systems are currently being examined. This will indorse the correlation between enzymatic activity and membrane structure influencing the regulation of physiological processes.

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Protein-Lipid Interactions Shaping the Electrostatic Membrane Search of a Pleckstrin Homology Domain

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The membrane-targeting domains of peripheral proteins play an important role in mediating cell signaling events originating at the plasma membrane. The pleckstrin homology (PH) domain is the most common membrane targeting domain, and many PH domains specifically recognize membrane-bound PIP lipids. Recently, the representative PH domain of the General Receptor for Phosphoinositides (GRP1 PH) has been found to use an electrostatic search mechanism requiring anionic background lipids of the plasma membrane to more rapidly and tightly bind the rare phosphatidylinositol-3,4,5-phosphate (PIP3) lipid second messenger. The contributions of the seven basic residues on the GRP1 PH membrane-proximal face to the protein-lipid interactions that occur during electrostatic searching were investigated. Point and double mutants of the isolated Grp1 PH domain were purified with alanine replacing each of the seven basic residues. For each mutant domain, the relative affinities for phosphatidylinositol-3,4,5-trisphosphate (PIP3) were determined in the presence and absence of anionic background lipids. While the wild-type PH domain displays a ~10-fold enhanced affinity for PIP3 in the presence of anionic background lipids, this enhancement is significantly decreased in the point and double mutant PH domains possessing the R322A and K279A mutations. Thus, the results suggest that while most basic residues interact with the membrane at a detectable level, the protein-lipid interactions between basic residues R322 and K279 are most crucial for electrostatic searching. Additional experiments are in progress to determine the specificity of these protein-lipid interactions, and the effect of mutations on membrane binding kinetics.