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Condensing And Fluidizing Effects Of Structurally Related Gangliosides On Phospholipid Films

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In model membrane mixtures that mimic lipid raft compositions, the more ordered domains are enriched in the ganglioside, G_{M1}, which contains four neutral sugars and a negatively charged sialic acid. To understand the organization and partitioning of G_{M1} in cell membranes, the outer leaflet of the cell membrane was modeled using Langmuir monolayers of DPPC and varying concentrations of G_{M1}. At low biologically relevant concentrations, G_{M1} condenses the DPPC monolayer while at higher concentrations, it acts to fluidize, with a switch-over point between the two behaviors at a ratio of 3:1 DPPC:G_{M1}. To examine phase morphology and organization of the components, the monolayers were transferred onto solid substrates and imaged with atomic force microscopy. At concentrations below the switch-over point, G_{M1} is located in nanoscale clusters within the condensed DPPC domains. The total surface area of these nanosize domains is larger than that attributable to G_{M1} molecules alone, suggesting the regions are due to G_{M1} and DPPC packing preferentially in condensed geometric complexes.

To pinpoint the structural region of G_{M1} giving rise to the condensation effect, parallel experiments were run with ceramide and PEGylated lipids. Our results indicate that the bulky, rigid sugar ganglioside headgroup is necessary for the significant phase behavior effects on the surrounding lipid molecules. Ganglioside headgroup geometry and charge were further explored with binary mixtures of asialo-, disialo- and trisialo-gangliosides (containing zero, two, and three sialic acids) with DPPC. In all cases, a similar condensing and fluidizing effect that varies with ganglioside concentration is seen, suggesting the negatively charged sialic acid residue is not critical for the close-packing phenomenon. Variations in mole ratio of critical packing between different ganglioside molecules can be explained by global effects of headgroup geometry, charge, and resultant molecular dipole moments.

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Nanoscopic Rearrangement Of Outer And Inner Leaflet Membrane Proteins Due To Ige Receptor Cross-linking

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Antigen-mediated cross-linking of immunoglobulin E (IgE) bound to its high affinity receptor FcεRI on mast cells initiates a transmembrane signaling cascade that results in cell activation and exocytotic release of chemical mediators involved in allergic response. Plasma membrane lipids and proteins redistribute as part of this transmembrane signaling process. To understand the functional role of these redistributions, resolution of their size, composition and structure on the nanometer scale is required. We utilize high resolution scanning electron microscopy (SEM) to directly visualize sub-micron membrane domains in intact cell membranes. In our experiments, the distribution of gold-labeled proteins and lipids is analyzed at the surface of intact fixed cells using backscattered electron detection. In parallel, we also observe membrane topography using secondary electron detection. We use a pair-correlation function analysis to quantify protein distributions and parameterized domain size. We have mapped the distribution of a variety of proteins, both related and non-related to the IgE signaling pathway. Using this experimental and quantitative method, we observe dramatic changes in the nano-scale membrane distribution of IgE due to stimulation with multivalent ligands. In resting cells, IgE receptors are clustered into small domains of less than 30nm. Following receptor cross-linking, receptors are rapidly redistributed into large domains which are correlated at long length-scales. Additionally, we observe cross-linking dependent rearrangement of several inner leaflet-associated proteins that are implicated in early signaling events. In contrast, outer leaflet GPI-linked proteins are not affected. These findings demonstrate selective nanoscopic reorganization during the initiation of receptor signal transduction.

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Cholesterol Content And Domain Formation As Regulators Of PLA2-IIA Activity In Anionic Membranes

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Phospholipase A_2 type IIA (PLA_2-IIA) hydrolyses the sn-2 position of glycerolipids to produce free fatty acids and lysolipids. The enzyme presents a strong affinity towards membranes enriched in anionic lipids, and lipid-packing is known to influence the ability of PLA_2-IIA to extract lipids from the membrane. This leads to an interrelation between membrane structure, and enzyme activity. In this study we evaluate the activity of PLA_2-IIA on unilamellar vesicles composed of either 1- palmitoyl-2-oleoyl phosphatidylglycerol (POPG) or dimyristoyl - phosphatidylglycerol (DMPG), in combination with cholesterol (Chol) and sphyngomyelin (SM). We expose the vesicles to PLA_2-IIA and monitor the activity at 37°C, where both POPG and DMPG are in the liquid-disordered phase in their pure form. For DMPG/Chol or POPG/Chol the results show that adding cholesterol alone inhibits PLA_2-IIA activity. However, this effect is more accentuated in DMPG vesicles compared to POPG vesicles. We attribute this difference to a closer interaction of cholesterol with the saturated acyl chains of DMPG, leading to tighter lipid packing and a reduced hydrolysis rate. In the second part of the study, for POPG and DMPG samples in which we included cholesterol and sphyngomyelin in equal molarities, we detect high hydrolytic activity in a wider range of compositions for a given Chol/PG ratio compared to samples without SM. We propose that a strong affinity between SM and cholesterol, related to liquid-ordered domain formation, leads to a depletion of cholesterol from the PG-rich regions, maintaining a high PLA_2-IIA hydrolysis rate. In the third part of our study, we focus on changes in rigidity of the membranes after exposure to PLA_2-IIA based on Laurdan General Polarization measurements. We find a general increase in rigidity following the hydrolytic burst in POPG binary and ternary systems.

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Comparison of Insertion and Folding of Chaperone-bound Outer Membrane Protein A (OmpA) of E. coli into Phospholipid Bilayers of Various Composition

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OmpA spontaneously inserts and folds into lipid bilayers from a urea-unfolded state upon urea-dilution. Previous work demonstrated that urea can be replaced effectively by the periplasmic chaperone Skp when lipopolysaccharide (LPS), a component of the outer membrane, is present [1]. Skp was shown to bind outer membrane proteins with nanomolar affinity and to prevent their aggregation [2].

Here we investigated folding of Skp-bound OmpA into lipid bilayers of different headgroup composition and chain-length, both in absence and presence of LPS. For urea-denatured OmpA and in absence of Skp and LPS, kinetics of folding into bilayers containing the negatively charged phosphatidylglycerol showed a lag-phase of up to 30 min for dilauroylphospholipid bilayers (LUVs, 100 nm diameter) prior to folding. Skp inhibited folding and prolonged the lag-phase at basic pH when LPS was absent. In presence of LPS, no lag-phase was observed and folding rates increased dramatically.

When similar experiments were performed with bilayers composed of the corresponding dioleolylphospholipids (SUVs), a lag-phase was not observed, but Skp inhibited very strongly. LPS again stimulated OmpA insertion and folding, albeit not as much as observed for dilaurylphospholipid bilayers.

Skp inhibited folding also in experiments with neutral phosphatidylcholine bilayers, irrespective of lipid chain-length. Here, LPS could also facilitate folding of Skp-bound OmpA, but the effect was less pronounced for dioleoylphosphatidylcholine bilayers. The data suggest, LPS-assisted folding of Skp-bound OmpA depends on both, the surface charge of the membrane and on the lipid-chain composition.

References:
1. Bulieris, P. V., et al. (2003). J Biol Chem 278, 9092-9.
2. Qu, J. et al. (2007). J Mol Biol 374, 91-105.

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Binding Sites of Outer Membrane Protein A (OmpA) in the Complex with the Periplasmic Chaperone Skp from E. Coli. A site-directed fluorescence study

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The periplasmic chaperone Skp facilitates folding and insertion of membrane proteins into the outer membrane of Gram-negative bacteria [1,2]. We have studied the binding sites of OmpA in complex with Skp or with Skp and LPS [3] in aqueous solution by site-directed mutagenesis and fluorescence spectroscopy. Single tryptophan mutants of OmpA were prepared and isolated in unfolded form in 8 M urea solution. In thirteen mutants, the single tryptophan was introduced at different positions, namely in 5 of the 8 β-strands, in the 4 outer loops, and in the 3 turns of the 170 residue transmembrane domain and in addition in the 155 residue periplasmic domain. All mutants folded upon