Membrane Structure I

2310-Pos Board B280

Molecular Dynamics Study of Cermide:POPC mixtures

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Ceramide (Cer) is the simplest lipid in the biologically important class of glycosphingolipids. Ceramide is an important signaling molecule and a major component of the strateum corneum layer in the skin. In order to begin to understand the biophysical properties of ceramide, we have carried out a Molecular Dynamics simulations of mixtures of hydrated 16:0-ceramide:POPC lipid bilayers at temperatures 323K and 368K. These simulations consist of 5%,10%, 15%, 20%, 30%, 40%, 60%, 80%, and 100% Cer in POPC bilayers. We computed electron densities and order parameters to investigate the structural properties of these mixtures. We compared our simulation results with the experimental phase diagrams of Cer:POPC mixtures.

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The Physical Properties of Sphingomyelin/Cholesterol Membranes: a Deuterium NMR Study to Map a Partial Phase Diagram

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We have used deuterium nuclear magnetic resonance (^{2}H NMR) to study the effect of cholesterol on N-palmitoyl(D31)-D-erythro-sphingosylphosphorylcholine (PSM) membranes. NMR spectra were taken as a function of temperature (from 25 to 70°C) and cholesterol concentration (0 - 40%). The constructed phase diagram exhibits both solid-ordered (**so**) + liquid-ordered (**lo**) and liquid-disordered (**ld**) + **lo** phase coexistence regions with a clear three-phase line at 37.5°C between 8 and approx. 18 mol% cholesterol. The **ld** + **lo** region was characterized by examining the cholesterol dependence of the width of resolved peaks in the depaked spectra, as well as the average spectral width (M_1), at a given temperature. The **so** + **lo** region was defined using spectral subtraction. Analogous experiments were done using 1-palmitoyl,2-palmitoyl(D31)-*sn*-glycero-3-phosphocholine (DPPC)/cholesterol membranes in order to carefully compare the data obtained using palmitoyl chains which have similar ''kinked'' conformations. The three-phase line in sn-2 perdeuterated DPPC/cholesterol is at 39.5°C and extends from approx. 8 to 20 mol% sterol. The PSM/cholesterol membrane is significantly more ordered than the DPPC/cholesterol membrane in the liquid crystalline phase. For example, at 60°C and 20 mol% cholesterol, the average order parameter of PSM is 34% higher than for DPPC. This should be compared to the difference between the cholesterol-free membranes at 60°C, where the average order of PSM is 23% more than that of DPPC. However the PSM/chol interaction is more effective than that of DPPC/chol at removing conformational fluctuations in the palmitoyl chain.

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Inositol Phosphorylceramide - Characterization of membrane properties and their calcium dependence

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We have characterized the membrane properties of inositol phosphorylceramide (IPC) in model membranes with emphasis on calcium induced effects. IPCs are a class of anionic sphingolipids with a single phosphoinositol head group coupled to ceramide. IPCs have been identified in eukaryotic species and also observed in detergent resistant membranes in *Leishmania major*. Here we report on the properties of *N*-palmitoyl-IPC (PIPC) in one component bilayers as well as in complex bilayers together with POPC and sterols. According to anisotropy changes reported by DPH and initial DSC experiments, the gel-to-liquid main transition temperature (Tm) of PIPC is about 50 °C. Addition of 5 mM Ca^{2+} during vesicle preparation markedly increased the Tm to about 65 °C. DPH anisotropy also showed that cholesterol (at 20 mol %) was able to decrease the order in the gel-phase and increase the order in the liquid disordered-phase. Fluorescence quenching studies using the fluorescent sterol analogues cholestatrienol or dehydroergosterol and the doxyl labeled lipid 7SLPC as quencher, showed that PIPC was able to form sterol-enriched ordered domains in the otherwise fluid bilayer environment. Dynamic light scattering studies indicate a clustering of PIPC containing SUVs (PSM:PIPC, 8:2) due to addition of Ca^{2+} to a final concentration of 5 mM. The increase in Tm and apparent clustering of PIPC containing vesicles shows that PIPC/Ca^{2+} interactions have substantial effects on the behavior of PIPC in model membranes. IPCs and the calcium induced effects could be important in membrane associated cellular processes such as membrane fusion and membrane raft linked processes.

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Ceramide-Enriched Membrane Domains in Red Blood Cells and the Mechanism of Sphingomyelinase-Induced Hot-Cold Hemolysis

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Hot-cold hemolysis is the phenomenon whereby red blood cells, preincubated at 37 degrees C in the presence of certain agents, undergo rapid hemolysis when transferred to 4 degrees C. The mechanism of this phenomenon is not understood. PlcHR 2, a phospholipase C/sphingomyelinase from Pseudomonas aeruginosa, induces hot-cold hemolysis. We found that the sphingomyelinase, but not the phospholipase C activity, is essential for hot-cold hemolysis because the phenomenon occurs not only in human erythrocytes that contain both phosphatidylcholine (PC) and sphingomyelin (SM) but also in goat erythrocytes, which lack PC. Fluorescence microscopy observations confirm the formation of ceramide-enriched domains as a result of PlcHR 2 activity. After cooling down to 4 degrees C, the erythrocyte ghost membranes arising from hemolysis contain large, ceramide-rich domains. We suggest that formation of these rigid domains in the originally flexible cell makes it fragile, thus highly susceptible to hemolysis. We also interpret the slow hemolysis observed at 37 degrees C as a phenomenon of gradual release of aqueous contents, induced by the sphingomyelinase activity. These hypotheses are supported by the fact that ceramidase, which is known to facilitate slow hemolysis at 37 degrees C, actually hinders hot-cold hemolysis. Differential scanning calorimetry of erythrocyte membranes treated with PlcHR 2 demonstrates the presence of ceramide-rich domains that are rigid at 4 degrees C but fluid at 37 degrees C. Ceramidase treatment causes the disapperance of the calorimetric signal assigned to ceramide-rich domains. Finally, in liposomes composed of SM, PC, and cholesterol, which exhibit slow release of aqueous contents at 37 degrees C, addition of 10 mol % ceramide and transfer to 4 degrees C cause a large increase in the rate of solute efflux.

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Influence Of Ganglioside GM1 On Formation And Properties Of Rafts In Lipid Membranes

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Gangliosides are the universal components of membranes of eukaryotic cells playing a number of important regulatory functions. It has been previously demonstrated that release of certain gangliosides from malignant cells can be responsible for inducing apoptosis of immunocompetent cells. One of the possible mechanisms includes raft formation, oligomerization of pro-apoptotic receptors with consequential apoptosis signal transduction. In particular, GM1 and GM3 can both form their own microdomains and partition to rafts and caveolae in membranes. Since investigation of raft properties in living cells is greatly complicated by small size of rafts, and other factors, giant unilamellar vesicles (GUV) made of biological membrane mimicking mixture of lipids were used as a model system.

Fluorescence confocal microscopy was employed to study distribution of ganglioside GM1 in GUV and its influence on formation and properties of rafts in bilayer lipid membranes. Both raft and non-raft phase markers were added to membrane-forming lipid mixture. No visible phase separation was observed without GM1 unless lateral tension was applied to the membrane by osmotic stress. At 2 mol% concentration of GM1, large domains appeared indicating macroscopic phase separation. Increase of GM1 content to 5 mol% resulted in transformations of the domain shape consistent with significant growth of line tension at the domain boundary. On further increase of GM1 concentration to 10 mol%, almost all domains were pinched out from the vesicles, forming their own homogeneous liposomes. Estimates indicated that changing GM1 content from 2 mol% to 5-10 mol% resulted in the increase of the line tension at domain boundary by several hundred percent.