compared to those containing C_{16}-ceramide. Work supported by funding from the Sigrid Juselius Foundation (JMH), the Finnish Cultural Foundation (JMH), Evald and Hilda Nissi Foundation (JMH), The Finnish Eye Foundation (JMH), the Academy of Finland (AH, IV, SW), and from the Natural Sciences and Engineering Research Council of Canada (MRM).

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Sterol Solubility in Vesicles (GUV's) Containing the Ternary Lipid Mixture DPPC:DOPC:Sterol by Quantitative NMR

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In the laboratory, giant unilamellar vesicles (GUVs) offer a rich system for studying miscibility of cholesterol and phospholipids in a lipid bilayer. Our laboratory produces vesicles through various methods, but this study will focus on electroformation. Our work and the work of many other laboratories rely on the assumption that the electroformation process creates vesicles with the same lipid composition originally assembled in the electroformation chamber, up to an ultimate sterol solubility limit. A few sterol solubility limits have been previously tested in selected binary systems [1], but are not generally known for ternary systems. It is important for us to test our assumption that the electroformation process does not significantly alter lipid composition, and also to determine the solubility limit of sterols in membranes in order to understand and properly present the results of other GUV studies from our laboratory. Here we describe studies using quantitative NMR to ascertain the solubility of sterols for which convenient chemical assays exist (e.g. cholesterol) and for which chemicals assays are not readily available (e.g. ergosterol).

[1] Huang J., Buboltz J.T., Feigenson G.W., 1999. Maximum Solubility of Cholesterol in Phosphatidylcholine and Phosphatidylethanolamine Bilayers. Biochim. et Biophys. Acta 1417, 89-100.

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Modeling The Temperature Dependence of Membrane Solubilization by Detergents

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It is known that lipid membranes become, typically, less susceptible to solubilization with increasing temperature so that more detergent is needed to start and complete their conversion to mixed micelles. Qualitatively, this can be explained by the fact that thermal chain disordering and headgroup dehydration render the spontaneous curvature of the molecules less positive.

Here we present an alternative model to describe this temperature dependence quantitatively in terms of simple, physically meaningful model parameters. This model relates the onset of solubilization to the detergent concentration when micellization becomes more favorable than membrane insertion. It quantifies the effect of temperature on membrane versus micellar packing effects in terms of the heat capacity changes of partitioning (-0.75 kJ/(mol K)) being more negative than that of micelle formation (-0.52 kJ/(mol K)). We demonstrate the model based on measurements of the CMC, partition coefficient and heat capacity changes for pentaethylene glycol monodecyl ether (C10E5) interacting with membranes of POPC by isothermal titration calorimetry (ITC).

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An Approximate Cooperativity Analysis By Dsc And Uv-vis Phase Of Pseudobinary DXPC-DC8,9PC-Cholesterol Dispersions

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The effect of DC8,9PC on the cooperativity of ordered-fluid phospholipid phase transitions has been investigated by determining the transitions widths in multilayer's dispersions of DXPC - DC8,9PC by DSC. The van' t Hoff values of the main transition enthalpies were calculated using an approximate expression deduced from Zimm and Bragg theory. The DC8,9PC decreases the cooperativity (size of the cooperative unit of synthetic DXPC bilayers). The observation that the membrane lipids in the mixed lipid/DC8,9PC assemblies appear to adopt bilayer structures is important, since it demonstrates that the lipid domains within the colorimetric vesicles exist in the fundamental organizational unit found in cellular membranes. The experiments described in this work shed light upon the effects of external environmental parameters, such as temperature, upon structural and dynamical properties of the organized lipid assemblies. The colorimetric platform also facilitates elucidation of the contribution of distinct membrane components, such as cholesterol, toward shaping membrane properties. This capability could open the way for application of the assay for detailed analyses of the roles played by particular molecules, such as peptides and DNA, in determining membrane functions and properties. Between non polymeric and polymeric species in DXPC: DC8,9PC mixtures the influence of the polymer is from C14 chain-down more cooperative the non-polymeric; from C16 on before DPPC:DC8,9PC is better polymerized than non-polymerized and for DSPC:DC8,9PC there is no difference. Hypothesis is C14 or less non-polymerized are intermixed with no difference but when polymerized, non ideal mixing is formed, polymers containing polymeric units with pockets containing DMPC and/or cholesterol. When chains are C16 or more units of saturated and non saturated mixtures cooperative units are quite similar.

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Energetics of Cholesterol Transfer between Lipid Bilayers

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It is believed that natural biological membranes contain domains of liquid ordered phase enriched in cholesterol and sphingomyelin. Although the existence of these domains, called lipid rafts, is still no firmly established for natural membranes, direct microscopic observations and phase diagrams obtained from the study of three-component mixtures containing saturated phospholipids, unsaturated phospholipids, and cholesterol demonstrate the existence of lipid rafts in synthetic membranes. The presence of the domains or rafts in these membranes is often ascribed to the preferential interactions between cholesterol and saturated phospholipids, for example, between cholesterol and sphingomyelin. We calculated, using molecular dynamics computer simulation technique combined with the umbrella sampling and weighted histogram analysis method (WHAM), the free energy of cholesterol transfer from the bilayer containing unsaturated phosphatidylcholine lipid molecules to the bilayer containing sphingomyelin molecules and find that the affinity of cholesterol to sphingomyelin is higher. By doing the simulations at different temperatures, we calculated the free-energy components, energy and entropy, and show that cholesterol transfer is exothermic and with a loss of entropy. The transfer is promoted by the favorable change in the lipid-lipid interactions near cholesterol and not by the favorable energy of cholesterol-sphingomyelin interaction, as assumed previously.

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Impact Of Ceramide3 On POPC Host Membranes: A Study On Structure And Thermodynamics

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The uppermost layer of skin epidermis exhibits a very peculiar lipid composition consisting mostly of long-chain ceramides of asymmetric chain length. The functional impact of ceramides on 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membranes was investigated using natural phytosphingosine type ceramide3 (Cer3) as a model system. Results will be presented on structure and thermodynamic phase behavior of the composite model system for three mole ratios Cer3/POPC (5:95; 10:90; 15:85). All methods applied so far (SAXS; DSC; confocal microscopy) reveal non-ideal miscibility of the two compounds with macroscopic separation of coexisting lamellar phases of different rigidity. Two major transition regions were identified: at low temperature(T_{m1}) and at high temperature(T_{m2}). They were attributed to POPC rich and Cer3 rich domains respectively. A slight increase in the d-spacing and as well a shift of T_{m1} towards higher values indicate a solidifying effect of Cer3 on POPC host membranes. At low concentration the Cer3 rich domain (T_{m2}) exhibits normal thermodynamic behavior with freezing point depression due to the presence of POPC. The continuous shift in T_{m1} together with a loss of transition cooperativity for the POPC rich domain hints towards a modification of the bilayer elasticity due to the presence of the Cer3. The overall heat content of the composite system increases with the amount of Cer3 present, signifying a stronger attractive interaction among the lipids. The Cer3 rich phase is almost dehydrated. Giant vesicles (GUVs) exhibit fluid-gel domain coexistence with the typical smooth and flat face facets as signatures of the more fluid and the more rigid membrane regions.

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The Effect Of Glycerol On Membrane Solubilization By Nonionic Surfactants

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Some protocols for solubilizing membrane components by surfactants use glycerol as a co-solute for stabilizing the native state of proteins. We have studied