liquid-ordered domains (rafts) that exist in the intact membrane prior to the addition of TR, the cause of formation of DRM is not fully understood. Our working hypothesis was that the formation of DRM is due to the established strong binding of SM to cholesterol. This results in the formation of SM=cholesterolcomplexes with a large negative spontaneous curvature, which stabilizes it against solubilization. This interpretation implies that under conditions of incomplete solubilization the solubilized (micellar) fraction arising from bilayers composed of PC and SM, will be rich in SM whereas in cholesterol-containing mixtures, the solubilized fraction will be rich in PC. To test these predictions, we have determined the SM/PC ratio in mixed micelles, using high resolution ^{31} P-NMR, to which non-solubilized phospholipids do not contribute, because of being broadened beyond detection. The results were as expected, thus supporting our working hypothesis (SM/PC$>$ 1.0 in the solubilized fraction of SM-PC liposomes; SM/PC$>$ 1.0 in the solubilization of liposomes made of PC, SM and cholesterol), This supports the hypothesis that the detergent-resistance of SM is due to the relative stability and large negative spontaneous curvature of the SM-cholesterol complexes. The results also demonstrate the strength of ^{31}p-NMR spectroscopy in the investigation of the partial solubilization of specific membrane phospholipids without having to separate the solubilized from non-solubilized fractions.

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Simple Phenomenological Model and Phase Behavior of Ternary Mixtures of Saturated and Unsaturated Lipids and Cholesterol

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We present a phenomenological theory for the phase behavior of ternary mixtures of cholesterol and saturated and unsaturated lipids, one which describes both liquid and gel phases. It leads to the following description of the mechanism of the phase behavior. In a binary system of the lipids, phase separation occurs when the saturated chains are well ordered, as in the gel phase, simply due to packing effects. In the liquid phase the saturated ones are not sufficiently well ordered for separation to occur. The addition of cholesterol, however, increases the saturated lipid order to the point that phase separation is once again favorable. Our theory addresses this last mechanism, the means by which cholesterol-mediated ordering of membrane lipids leads to liquid-liquid immiscibility. It produces, for the system above the main chain transition of the saturated lipid, phase diagrams in which there can be liquid-liquid phase separation in the ternary system but not in any of the binary ones, while below that temperature it yields the more common phase diagram in which a gel phase, rich in saturated lipid, appears in addition to the two liquid phases.

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Cholesterol-phospholipid Interactions: New Insights From Surface X-ray Scattering Data

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In cell membranes, cholesterol-enriched domains are presumably involved in a wide variety of cellular processes. Although a number of conceptual models exist, there is no consensus on the molecular mechanism of cholesterol-phospholipid interactions. Here we report on a systematic study of cholesterol-phospholipid interactions in lipid monolayers using Langmuir isotherms, epifluorescence microscopy, synchrotron X-ray reflectivity (XR), and grazing incidence X-ray diffraction (GIXD) techniques. Lipid monolayers consisted of cholesterol-DPPC mixtures with various cholesterol mole fractions (χCHOL from 0 to 1). XR results demonstrate that cholesterol tends to stay in the acyl chains region of DPPC with its hydroxyl group in a proximity to carbonyl groups of the phospholipid. Increase in cholesterol content promotes ordering of the phospholipid acyl chains. Moreover, X-ray and Langmuir isotherm data used in a complimentary manner indicate that in cholesterol-lipid mixture cholesterol molecule craves to grab an additional 10 Å2 of molecular area from the acyl chains directly above the phospholipid headgroups. These results provide a reasonable explanation for the well documented ''condensing effect'' of cholesterol in lipid mixtures. At high cholesterol concentrations the phospholipid headgroups tilt significantly, but even then appear to be incapable of providing an additional 10 Å2 required to enclose the cholesterol molecules. Interestingly, the critical cholesterol concentration at which phospholipids still shield cholesterol molecules is the same as that at which the phase transition from α- to β-region observed with the epifluorescence microscopy. GIXD data yield DPPC crystalline order only in the mixtures with χCHOL below 0.15. At higher χCHOL, cholesterol seizes the places of the acyl chains in the DPPC crystalline lattice at the same stoichiometry as cholesterol and DPPC in the mixture. Diffraction pattern of such mixtures yields a short-range hexagonal packing order with d-spacing increasing as a function of the χCHOL.

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The Physical Properties Of Model Membranes Containing POPC, POPE And Sterol: A Deuterium NMR Study

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We have investigated the effect of sterol on the physical properties of lipid membranes containing 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE). POPC/POPE/ergosterol, POPC/POPE/cholesterol, and POPC/POPE membranes were studied as a function of temperature using deuterium nuclear magnetic resonance (^{2}H NMR), with POPC and POPE deuterium labeled alternatively. It is found that the presence of ergosterol or cholesterol disorders gel-phase POPC/POPE (1:1) membranes, whereas orders lc-phase membranes. The modulation of lipid orders by ergosterol is less dramatic than that by cholesterol. In addition, the presence of ergosterol or cholesterol modulates the interaction between POPC and POPE. It is of interest that in POPC/POPE/cholesterol both lipid components display identical M_1(T) curves, which does not observed in POPC/POPE/ergosterol and POPC/POPE.

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Effects of Cholesterol and Unsaturated DOPC Lipid on Chain Packing of Saturated Gel-phase DPPC Bilayers

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Wide angle x-ray scattering (WAXS) from oriented lipid multilayers was used to study the effect of adding cholesterol (Chol) or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to gel-phase 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers. Small quantities (X<0.10 mole fraction) of both molecules disrupt the tight packing of tilted chains of pure gel-phase DPPC, forming a more disordered, untilted phase. The addition of larger quantities of DOPC causes the sample to phase-separate into a gel phase, characterized by a narrow WAXS peak, and liquid disordered phase, characterized by wide, diffuse WAXS scattering. In contrast, two WAXS peaks indicative of two coexisting phases were not observed in Chol/DPPC mixtures (X_{Chol} = 0.07 to 0.40). Instead, Chol caused a gradual increase in the width of the WAXS peak, consistent with a gradual change from a more gel-like to a more liquid-like state rather than passing through a region of two phase coexistence. Our WAXS data include a huge amount of information. A new method of analysis suggests that WAXS data may provide definitive results relating to the disagreements between previously published phase diagrams for Chol/DPPC.

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Role Of Membrane Cholesterol Content In The Activity Of Cyclooxygenase-2 (COX-2) In MCF-7 Human Breast Cancer Cells

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Cyclooxygenase-2 (COX-2) and its product PGE_2 are known to increase both angiogenesis and resistance to apoptosis (promoting tumor growth) and to enhance the penetration of cancer cells into adjacent tissues (causing metastasis). Thus, knowing how the activity of COX2 is regulated at the cellular level has implications for breast cancer therapeutic strategies. The goal of this research is to unravel a new molecular mechanism for regulating the activity of COX-2. The proposed molecular mechanism may be elucidated by using the sterol superlattice model. In plasma membranes, arachidonic acid (AA) is released by phospholipase A2 (PLA2). Cyclooxygenase then converts AA to prostaglandins (e.g., PGE_2). The activity of PLA2 is known to vary with membrane cholesterol content in an alternating manner, showing a local minimum at critical sterol mole fractions (C_r) for maximal superlattice formation. Hence, it is logical to hypothesize that the activity of COX (including the isoform COX-2) also varies with cholesterol content in a biphasic manner. In this study, the cholesterol content in MCF-7 human breast cancer cells was decreased systematically by using methyl-beta-cyclodextrin. A biphasic change in COX-2 activity, as monitored by the Cayman COX-2 assay with minor modifications, was observed at certain cell cholesterol content C_{rcell}. The cholesterol content near C_{rcell} could serve as a fine-tuning mechanism to regulate COX-2 activity and PGE_2 production, and consequently, cancer cell growth and metastasis. (supported by DOD, NSF and PDOH)

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Free Energy Of Cholesterol Transfer In Lipid Bilayers With Varying Degree Of Saturation

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