

bilayer and therefore plays an important role in maintaining lipid asymmetry. Leaflet composition is regulated by the active transport of lipids by membrane proteins, while thermal diffusion across a membrane tries to randomize the leaflet composition. We have measured the transbilayer diffusion rates for three different sterols over a wide range of compositions. The sterols studied were all cholesterol analogs; including dihydrocholesterol, ergosterol, a component of fungal cell membranes, and stigmasterol, an unsaturated plant sterol. Temperature was varied to determine its influence on transbilayer diffusion rates. We find that sterol structure does have an influence on the rate at which lipids move between bilayer leaflets. Transbilayer diffusion measurements were made using a sodium dithionite assay to monitor the location of lipid analogues within DMPC/sterol liposomes.

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Cholesterol Flip-flop And Chemical Potential In A Systematic Set Of Lipid Bilayers

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Cholesterol is a necessary component of animal cellular membranes. The concentration of cholesterol varies from 0-5 mol% in the endoplasmic reticulum to 25-40mol% in the plasma membrane. Thermal fluctuations cause cholesterol to move normal to the plane of the bilayer. At the extremes, cholesterol can translocate across the bilayer (flip-flop) and diffuse from the bilayer into water (desorption). We have used atomistic and coarse grained molecular dynamics computer simulations to investigate the partitioning of cholesterol through a systematic set of lipid bilayers. Atomistic simulations provide detailed analysis, while inexpensive coarse grained simulations allow more bilayers to be investigated and longer time scales to be sampled. From the coarse grained simulations, cholesterol flip-flop was directly observed, and the rate matched our estimate from the free energy barrier. We find the rate of cholesterol flip-flop is fast and strongly dependent on the structure of the bilayer. The rate of flip-flop is on the microsecond range in fluid, disordered poly-unsaturated bilayers, and on the second range in rigid, ordered bilayers with high cholesterol content. The chemical potential of cholesterol in the bilayer compared to water is equal to our free energies of desorption. We can infer the relative affinity of cholesterol for the bilayers by comparing the chemical potentials. We find cholesterol prefers more ordered and rigid bilayers with saturated acyl tails, and high cholesterol content. Cholesterol has the lowest affinity for poly-unsaturated lipids.

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The Behavior of Two Oxidized Derivatives of Cholesterol in Model Membranes

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Cholesterol's role in ordering lipid membrane domains is well known. Even subtle changes in the structure of this sterol greatly affect the biophysical dynamics of membranes, usually because of perturbations in the interactions between the sterol and other membrane lipids that chemical modifications cause.

Cholesterol oxidation products (oxysterols), which result from enzymatic and non-enzymatic mechanisms, are cytotoxic and found in atherosclerotic plaques. Previous studies have shown that the membrane properties of oxysterols vary, depending on the specific site of the oxygen-containing moiety. In this study, we examined the interactions of two oxysterols, one formed through non-specific oxidation (7-ketocholesterol), and one produced enzymatically (25-hydroxycholesterol) with two common membrane lipids, 1-palmitoyl-2-oleoyl-*sn*-phosphocholine (POPC) and brain-derived sphingomyelin.

Analysis of force-area isotherms obtained by compression of pure sterol monolayers and of binary monomolecular films at the air-water interface, comprised of varying mole fractions of POPC or sphingomyelin and either oxysterol, reveals significant differences in surface behavior with respect to each other and to native cholesterol. Both oxysterols condensed POPC and sphingomyelin films to a lesser degree than cholesterol, and an expansion of sphingomyelin films was observed with low mole fraction 7-ketocholesterol. Additionally, surface compression moduli data obtained from the force-area isotherms reveal a decreased ability of both oxysterols to mitigate the phase transition of sphingomyelin compared to cholesterol. The changes of membrane behavior in the presence of oxysterols reported here suggest a relation of their toxicity to the propensity of lipids membranes to form liquid-ordered domains (rafts).

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A Calorimetric and Spectroscopic Comparison of the Effects on Ergosterol and Cholesterol on the Thermotropic Phase Behavior and Organization of Dipalmitoylphosphatidylcholine Bilayer Membranes

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We performed comparative DSC and FTIR spectroscopic measurements of the effects of cholesterol (Chol) and ergosterol (Erg) on the thermotropic phase behaviour and organization of DPPC bilayers. Erg is the major sterol in the biological membranes of yeasts, fungi and many protozoa. It differs from Chol in having two additional double bonds, one in the steroid nucleus at C7-8 and another in the alkyl chain at C22-23. Erg also has an additional methyl group in the alkyl chain at C24. Our DSC studies indicate that the incorporation of Erg is more effective than Chol is in reducing the enthalpy of the pretransition. At concentrations below 10 mol%, Erg is also more effective than Chol in reducing the enthalpies of both the sharp and broad components of main phase transition. However, at sterol concentrations from 30-50 mol %, Erg is generally less effective at reducing the enthalpy of the broad components and does not completely abolish the cooperative hydrocarbon chain-melting phase transition at 50 mol% as does Chol. Moreover, in this higher ergosterol concentration range there is no evidence of the formation of ergosterol crystallites or of the lateral phase separation of Erg-enriched phospholipid domains. Our FTIR spectroscopic studies demonstrate that Erg incorporation produces a less tightly packed bilayer than does Chol which is characterized by increased hydration in the glycerol backbone region of the DPPC bilayer. These and other results indicate that Erg is less miscible in DPPC bilayers at higher concentrations than is Chol.

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Schiff Base Formation Between The Cholesterol Oxidation Product 3 β -hydroxy-5-oxo-5,6-secocholestan-6-al And Amino Phospholipids

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The keto-aldehyde 3 β -hydroxy-5-oxo-5,6-secocholestan-6-al is formed by the oxidation of cholesterol with ozone. This oxidized form of cholesterol is associated with a number of pathological conditions including atherosclerotic plaques, Alzheimer's and Parkinson's diseases. We have shown earlier that the compound can react covalently with the amino group of phosphatidylethanolamine to form a Schiff base. Here, using a spectroscopic technique, we determine the kinetics of the Schiff base formation between 3 β -hydroxy-5-oxo-5,6-secocholestan-6-al and dimyristoylphosphatidylethanolamine in both the gel and liquid crystalline states of the phospholipid. The activation energies of this reaction in the two states are also calculated. In addition, we determine that a Schiff base can also be formed with the amino group of phosphatidylserine, albeit with slower kinetics. These findings are significant as they show that oxidized cholesterol can react covalently not only with the amino groups of proteins, but also with the amino groups of phospholipids, potentially influencing the structure of biological membranes.

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A Comparison Of Ceramide And Ceramide-1-phosphate Miscibility In Phosphatidylcholine Bilayers

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Sphingolipids are key lipid regulators of cell viability: ceramide is one of the key molecules in inducing programmed cell death (apoptosis), whereas other sphingolipids, such as ceramide 1-phosphate, are mitogenic. The phase behavior of bilayers comprising binary mixtures of N-hexadecanoyl-D-erythroceramide (C₁₆-ceramide) or N-hexadecanoyl-D-erythroceramide-1-phosphate (C₁₆-ceramide-1-phosphate; C₁₆-C1P) with 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) were studied using differential scanning calorimetry (DSC) and deuterium nuclear magnetic resonance (²H-NMR). Partial phase diagrams (up to a sphingolipid mole fraction of X=0.40) were constructed for both mixtures. For C₁₆-ceramide-containing bilayers DSC heating scans at X_{cer}=0.025 showed a complex structure of the main phase transition peak suggestive of lateral phase separation. The transition width increased significantly upon increasing X_{cer}, and the upper phase boundary temperature of the mixture shifted to ~65°C at X_{cer} = 0.40. The temperature range over which ²H-NMR spectra of C₁₆-ceramide/DPPC-*d*₆₂ mixtures displayed coexistence of gel and liquid crystalline domains increased from ~10° for X_{cer}=0.1 to ~21° for X_{cer}=0.4. DSC and ²H-NMR observations of C₁₆-C1P/DPPC mixtures at corresponding concentrations indicated that two-phase coexistence was limited to significantly narrower ranges of temperature for mixtures containing C₁₆-C1P