2544-Pos

α-Tocopherol and Polyunsaturated Fatty Acid Membrane Domians

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While α-tocopherol's role as the major membrane antioxidant has been certain for decades, its membrane structural role is far less certain. We are interested in a possible involvement of α-tocopherol in helping to hold proposed polyunsaturated fatty acid (PUFA)-rich, non-raft, domains together. The experiments presented here to test this hypothesis include differential scanning calorimetry (DSC), cold temperature detergent extractions and isothermal titration calorimetry (ITC) studies on model bilayer membranes and detergent extractions on living cell membranes. The DSC experiments indicate that in model membranes composed of sphingomyelin (SM) and 1-palmtioyl-2-docosahexaenoylphosphatidylethanolamine (16:0-22:6PE), cholesterol has a stronger association with SM while α-tocopherol prefers the polyunsaturated PE. Triton X-100 extractions on the same model system confirm that cholesterol segregates with SM (detergent insoluble fraction) and α-tocopherol segregates with 16:0-22:6PE (detergent soluble fraction). Currently underway are ITC experiments that measure the partitioning of α-tocopherol between lipid vesicles to quantify the stronger affinity for polyunsaturated lipids, and detergent extractions on living lymphocytes, where once again cholesterol is shown to preferentially segregate with SM into raft fractions, to observe preferential segregation of α-tocopherol with PUFA into non-raft fractions.

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Membrane Organization of Vitamin E is Sensitive to Lipid Unsaturation

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Vitamin E (α-tocopherol) has long been known as the major antioxidant in biological membranes. However, there remain many structurally related questions. Details of the molecular organization of α-tocopherol in membranes, for instance, lack the precision to address whether the vitamin has preferential affinity for unsaturated lipids in support of its role as an antioxidant. To observe how α-tocopherol interacts with unsaturated phospholipids, we determine, from one-dimensional neutron scattering length density profiles, the depth of deuterated analogs in phosphatidylcholine (PC) bilayers. The profiles obtained with α-[5-$^2H_3$]tocopherol and α-[9′-$^2H_2$]tocopherol in 1,2-dioloeylphosphatidylcholine (18:1-18:1PC) bilayers place the centers of mass of the labels 13 and 0 Å, respectively, from the bilayer center. They are consistent with the vitamin molecule sitting upright in the bilayer so that the hydroxyl group on the chromanol is near the aqueous interface and a highly disordered sidechain extends towards the middle of the membrane. The profile obtained for α-[5-$^2H_3$]tocopherol in 1-palmitoyl-2-oleeylphosphatidylcholine (16:0-18:1PC) reveals that, in contrast, the center of mass of the label sits 10 Å higher than in 18:1-18:1PC. A remarkable sensitivity upon membrane unsaturation for the depth of penetration of vitamin E is implied, and we are currently using solid state ^2H NMR and MD simulations to provide a detailed view of dynamical organization.

2546-Pos

Perturbation of Membrane Structure by Oxysterols

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Cholesterol is essential to the regulation and function of cell membranes, and cells expend large amounts of energy to control membrane cholesterol levels. The oxysterols, oxidation products of cholesterol, are enzymatically produced molecules that play a major role in regulating cholesterol homeostasis. Recent experimental work has shown that 25-hydroxycholesterol can affect cholesterol homeostasis through non-enantioselective mechanisms. Using molecular dynamics simulations, we have shown that cholesterol and 25-hydroxycholesterol alter membrane properties in very different ways, and that these effects are rooted in their orientations within the membrane. Newer simulations of bilayers containing both cholesterol and 25-hydroxycholesterol in the same membrane have shown that the presence of 25-hydroxycholesterol alters the position and orientation of cholesterol, increasing its solvent accessibility. Our work suggests that cholesterol and membrane perturbation by oxysterols may play a role in the oxysterol regulation of cholesterol homeostasis.

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Dynamics of Sedimentation and Deformation of GUVs Under Different Tonicity Conditions

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POPC Giant unilamellar vesicles (GUVs) with 1 mol% DiIC18(3) as a fluorescent marker were prepared by electroformation in sucrose solutions with varying osmolarities. These vesicles were resuspended in glucose solutions of different concentrations generating isotonic and hypertonic conditions. Vesicles sediment due to the density difference between the solutions. The movement of the vesicles as they approach the glass surface is studied using SPIM microscopy. We find that the velocity of the GUVs remains constant, given by Stokes law as expected when the distance from the surface is several radii in length. Velocity decreases exponentially as vesicles reach the surface. Vesicle deformation due to interactions with the surface was measured for different osmotic conditions using confocal and SPIM microscopy.

Boundary element simulations were performed to model vesicle deformation during sedimentation within a viscous fluid. For isotonic conditions, vesicles are assumed to begin with zero tension and tension is generated through contact with the surface. For the hypertonic case, the same is true but with an initial excess area available. Computationally, the mechanical behavior of the lipid bilayer is simulated using a model that considers two modes of deformation responsible for increases in area strain. The first is the smoothing of sub-optical thermal undulations and the second is the direct stretching of the area per lipid molecule. Properties of the lipid bilayer are controlled by adjusting bending and area compressibility moduli. A force field is implemented that takes into account local tension, local curvature force, and gravitational pull. Vesicle sedimentation, deformation, and membrane tension were evaluated as a function of g0, a dimensionless factor relating gravitational and curvature energies. Simulations are in agreement with the experimental results and provide additional information of the deformation of vesicles and sedimentation dynamics.

2548-Pos

Structure and Phase Behavior of Cholesterol Containing Membranes in the Presence of Ethanol

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Molecular dynamics (MD) of cholesterol containing membranes is used to examine the structural changes and phase behavior of lipids in the presence of varying ethanol concentrations over a range of temperatures. Alcohols are known to cause changes in the phase transitions of phospholipids as well as inducing the formation of an interdigitated phase of reduced thickness, where the hydrophobic tails of the top and bottom lipids intercalate causing an increase in the area per lipid as well as the solvent exposed surface of the headgroups. Atomistic MD simulations using the Gromacs 4.0 software allows analysis of structural changes in lipid volume, area per lipid, and hydrogen bonding among others at the molecular level. Pure 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) and DOPC/cholesterol membranes were constructed to have 128 lipid molecules under full hydration. DOPC/cholesterol systems contain 10, 20, and 30 mole % cholesterol, and those containing ethanol had additional 5, 10, 15 and 20 % V/V ethanol molecules in the solvent. All systems were simulated at 6 different temperatures which span relevant biological processes for biofuel production; also experimentally observed phase changes occur at some of these temperatures. The effects of ethanol on biological membranes are of considerable importance to the studies of biofuels, enology, and medicine.

2549-Pos

Miscibility Phase Behavior of GUV Membranes Containing Charge: Ternary Mixtures of Cholesterol, PC-Lipids, and PG-Lipids

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Giant unilamellar vesicles composed of a ternary mixture of phospholipids and cholesterol exhibit coexisting liquid phases over a range of temperatures and compositions. Few studies of phase behavior have been made using charged lipids, even though they account for a significant fraction of lipids in biological membranes. Here, I present phase diagrams of vesicles

containing phosphatidylcholine (PC), an uncharged lipid; phosphatidylglycerol (PG), a charged lipid; and cholesterol. These phase diagrams have several interesting features. Miscibility in membranes containing charged lipids occurs over similar ranges of temperatures and lipid compositions as in membranes containing only uncharged lipids. The coexisting liquid phases differ primarily in their phospholipid content such that one phase has a high concentration of charged lipid. Adding salt to the system causes an increase in transition temperatures at some membrane compositions, consistent with electrostatic screening, whereas the transition temperatures at other compositions fall.

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Activity and Ordering of Mixed Phosphatidylethanolamine/Dihydrocholesterol Monolayers

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Cholesterol is thought to be important for the structure and assembly of lipid rafts, and its interaction with other membrane lipids has been a topic of great interest. This study focuses on the interactions between 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) and dihydrocholesterol (Dchol) in Langmuir monolayers using fluorescence microscopy (FM), beta-cyclodextrin (CD) desorption assays, and grazing incidence x-ray diffraction (GIXD). Similar to our previous results for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)/Dchol monolayers [Biophys. J. 2007, 93, 2038-2047], FM and CD assays show 2 regimes for the DMPE/Dchol system. Short-ranged lateral ordering was observed using GIXD that was also consistent with our recent work on sphingomyelin (SM)/Dchol monolayers [Phys. Rev. Lett. 2009, 103, 028103]. We investigate how the smaller headgroup of DMPE affects the surface morphology, Dchol chemical activity, and lateral structure compared to monolayers of Dchol with DMPC or SM.

2551-Pos

Group III Secretory Phospholipase A_2 Enhances Alpha-Secretase-Dependent Amyloid Precursor Protein Processing Through Alterations in Membrane Fluidity

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Phospholipases A_2 (PLA_2s) are responsible for maintenance of phospholipids homeostasis in cell membrane and implicated in neurodegenerative disease including Alzheimer's disease (AD). Among many types of secretory PLA_2s, type III secretory PLA_2 ($sPLA_2$-III) is expressed in neuronal cells and contributes to cell differentiation and survival. Yet the role of $sPLA_2$-III in AD has not been explored. We studied the effects of $sPLA_2$-III and its hydrolyzed products, including arachidonic acid (AA), palmitic acid (PA) and lysophosphatidylcholine (LPC), on cell membrane fluidity in relations to amyloid precursor protein (APP) processing, which is an important cellular process in AD to produce either neuroprotective α-secretase-cleaved soluble APP ($sAPP_\alpha$) or neurotoxic amyloid-β peptide (Aβ). Differentiated human neuroblastoma (SH-SY5Y cells) treated with $sPLA_2$-III and AA, not PA and LPC, was found to increase $sAPP_\alpha$ secretion and these changes were accompanied by increased membrane fluidity and accumulation of APP at the cell surface. All the treatments altered neither total APP expression nor expression of α-secretases, including ADAM 9, 10, and 17. Taken together, our results support the hypothesis that $sPLA_2$-III enhances $sAPP_\alpha$ secretion through its action to increase membrane fluidity and recruitment of APP at the cell surface. This study provides insights into potential therapeutic approaches for AD treatment.

2552-Pos

Nonlinear Effect of Sucrose on Lamellar-Hexagonal Phase Transition Kinetics

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The topological nature of the lamellar-hexagonal phase transition in lipids makes it a useful tool in the study of pore formation. This phase transition in lipid-water systems is sensitive to the addition of various solutes such as sucrose. In our study of lipid SOPE (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine), we find that equilibrium lamellar-hexagonal phase transition temperature decreases linearly with sucrose concentration. However, we find that the phase transition kinetics vary in a strikingly nonlinear fashion. The speed of the transition greatly slows with even small concentrations of sucrose and then plateaus as the concentration increases.

2553-Pos

Structure of a DOTAP Lipid Bilayer: A Concerted Neutron Scattering and Molecular Dynamics Study

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Non-phospholipid, cationic 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) lipid based membranes fail to support the function of a voltage-dependent K+ channel due to the lack of a phosphate group (Schmidt et al., Nature 444, 775-779, 2006). However, the specific effects of the presence or absence of phosphate groups in the channel membrane environment on the voltage-sensing mechanism remain unknown. Before addressing the question of why DOTAP is not a suitable membrane environment for Kv channels, a detailed structural characterization of the pure DOTAP lipid bilayer system is required. Here, we employ molecular dynamics simulations in combination with neutron scattering experiments for a detailed atomistic study of a DOTAP lipid bilayer. All-atom molecular dynamics simulations of DOTAP bilayer at 9.4 waters/lipid were performed at constant pressure and temperature. One-dimensional structural data obtained from the neutron scattering experiments is used to validate the molecular dynamics simulations, which in turn provide the structural details at the atomistic level. We also compare the properties such as alkyl chain order parameter, area per lipid, and headgroup hydration, packing and orientation for a DOTAP lipid bilayer to zwitterionic phospholipid bilayers and propose the underlying physical-chemical reasons for the differences observed.

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2554-Pos

The Effect of Fatty Acids with Different Unsaturations on Membrane Fluidity and Alpha-Secretase-Dependent Amyloid Precursor Protein Processing

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Fatty acids are important dietary ingredients, which are implicated in neurodegenerative disease including Alzheimer's disease (AD). Yet their roles are not fully understood. We investigated the effects of fatty acids with different unsaturations (number of double bonds) including stearic acid (SA, 18:0), oleic acid (OA, 18:1), linoleic acid (LA, 18:2), α-linolenic acid (ALA, 18:3), arachidonic acid (AA, 20:4), eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6) on cell membrane fluidity in relations to amyloid precursor protein (APP) processing, which is an important cellular process in AD to produce either neuroprotective α-secretase-cleaved soluble APP ($sAPP_\alpha$) or neurotoxic amyloid-β peptide (Aβ). Differentiated human neuroblastoma (SH-SY5Y cells) treated with AA, EPA and DHA, not SA, OA, LA and ALA, increased $sAPP_\alpha$ secretion, which was accompanied by increased membrane fluidity. Our results showed that the fatty acids with four or more double bonds, including AA, EPA and DHA, promoted $sAPP_\alpha$ secretion through increasing membrane fluidity. This study provides the potential dietary strategies for the prevention of AD.

2555-Pos

PLA_2 Type IIA Increases Platelet Plasma Membrane Rigidity During Cold Induced Activation

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There is a wide discussion regarding the origin and function of lipid domains in human cells. An example in which lipid domains may play a functional role is the plasma membrane of human platelets. Platelets are susceptible to chilling, activating when the temperature falls below 20 °C. This limitation represents a crucial issue in terms of storage of platelets. It has been previously shown that cold-induced platelet activation is correlated with the formation of macroscopic lipid domains during cooling. This is a result of the fact that platelets present low cholesterol content (15 mol%), which results in the presence of a cooperative lipid melting transition centered at 15°C. This transition is responsible for the formation of the macroscopic lipid domains during cooling. Human secretory phopholipase A_2 type IIA ($sPLA_2$-IIA) catalyses the hydrolysis of the sn-2 ester bond in glycerolipids to produce fatty acids and lysolipids. Recently we have shown that its activity is triggered by the local enrichment of anionic lipids in fluid domains during phase coexistence. Since human platelets