be used to interact with model membrane to study their effect on chain-chain packing.

2539-Pos

Sterol Affinity for Bilayer Membranes is Affected by their Ceramide Content and the Ceramide Chain Length

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It is known that ceramides can influence the lateral organization in biological membranes. In particular ceramides have been shown to alter the composition of cholesterol and sphingolipid enriched nanoscopic domains, by displacing cholesterol, and forming gel phase domains with sphingomyelin. Here we have investigated how the bilayer content of ceramides and their chain length influence sterol partitioning into the membranes. The effect of ceramides with saturated chains ranging from 4 and 24 carbons in length was investigated. In addition, unsaturated 18:1- and 24:1-ceramides were also examined. The sterol partitioning into bilayer membranes was studied by measuring the distribution of cholestatrienol, a fluorescent cholesterol analogue, between methyl-beta-cyclodextrin and large unilamellar vesicle with defined lipid composition. Up to 15 mol% ceramide was added to bilayers composed of DOPC:PSM:cholesterol (60:20:20), and the effect on sterol partitioning was measured. Both at 23 and 37 °C addition of ceramide affected the sterol partitioning in a chain length dependent manner, so that the ceramides with intermediate chain lengths were the most effective in reducing sterol partitioning into the membranes. At 23 °C the 18:1-ceramide was not as effective at inhibiting sterol partitioning into the vesicles as its saturated equivalent, but at 37 °C the additional double bond had no effect. The longer 24:1-ceramide behaved as 24:0-ceramide at both temperatures. In conclusion, this work shows how the distribution of sterols within sphingomyelin-containing membranes is affected by the acyl chain composition in ceramides. The overall membrane partitioning measured in this study reflects the differential partitioning of sterol into ordered domains where ceramides compete with the sterol for association with sphingomyelin.

2540-Pos

Importance of Head Group Methylation on Sphingomyelin Membrane Properties and Interactions with Cholesterol

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Sphingomyelins (SMs) are important constituents of the plasma membrane and have, together with other sphingolipids and sterols, been identified as lipid components in membrane rafts. Interactions between sphingolipids and sterols have been shown to be important for the formation of ordered domains in model systems and also suggested to be a driving force in the formation of membrane rafts. The structure of sphingomyelins is important for interactions with sterols and in this study we have investigated the importance of the methyl groups in the head group of SMs upon membrane properties and interactions with cholesterol. Using specifically synthesized SM-analogues, having a stepwise decreasing number of methyl groups in the phosphocholine head group, we are able to systematically study how the size of the head group affects membrane properties and sterol interactions. The sphingomyelin analogues were composed of sphingosine having palmitic acid in the N-linked position. Using the anisotropy of 1,6-diphenylhexatriene we have determined the transition temperature between gel and liquid crystalline phases (Tm). Decreased methylation of the head group was shown to increase the Tm from 42°C (PSM), 53°C (dimethyl), 61°C (monomethyl) to 65 °C for ceramide-phoshoethanolamine (no methyl groups). Initial experiments using fluorescence quenching of cholestatrienol in complex model systems, shows that the ability to form sterol containing ordered domains also is affected by the degree of methylation in the head group. We are currently analyzing sterol interaction for the SM-analogues using DPH anisotropy and further prospects include determination sterol partitioning to vesicles containing each of the SM-analogues and differential scanning calorimetry studies.

2541-Pos

Spin Trapping the Oxidized Products of PUFA in Model Membranes: The Protection Conferred by Vitamin E

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Electron paramagnetic resonance (EPR) spectroscopy is recognized as the most sensitive and noninvasive means to quantify free radicals of biological relevance such as reactive oxygen species (ROS). In spin trapping a molecule (the spin trap) reacts with the free radical producing a spin adduct that is sufficiently stable to be detected by EPR. Here we apply a novel spin trapping technique to investigate the protection that α-tocopherol (vitamin E), the major lipid soluble antioxidant in membranes, confers on polyunsaturated lipids in model membranes. Polyunsaturated fatty acids (PUFA) readily oxidize because they have a *cis, cis*-1, 4-pentadiene motif that renders the central methylene group vulnerable to attack by ROS. Our method quantifies the oxidized products of PUFA in lipid vesicles that have been exposed to a physiologically relevant, oxidizing enzyme that initiates the free radical chain reaction. By measuring the reduction in lipid peroxidation due to the presence of α-tocopherol, we test the hypothesis that the vitamin co-localizes with polyunsaturated lipids in membrane domains to ensure close proximity to the most vulnerable lipid species.

2542-Pos

Cholesterol Displaces Ceramide from its Tight Packing with Sphingomyelin

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The present study deals with a combination of fluorescence spectroscopy, atomic force microscopy (AFM), differential scanning calorimetry (DSC) and confocal microscopy to study two different effects: i) lateral segregation in pSM/Chol binary mixtures, and ii) the effects of pCer incorporation into pSM/Chol mixtures. The data reveals the segregation of large cholesterol-enriched microdomains within the range X_{Chol} = 0-0.25 in the binary pSM/Chol mixtures. In comparison with the pSM/pCer mixture (Busto et al. 2009), sphingomyelin shows a higher preference for ceramide than for cholesterol. In ternary pSM/Chol/pCer mixtures, an immiscibility between cholesterol- (pSM/Chol) and ceramide-enriched (pSM/pCer) phases at high pSM/(Chol+pCer) ratio is observed, where no ceramide over cholesterol nor cholesterol over ceramide displacement is detected. Furthermore, the calorimetric and confocal microscopy data concur in showing an inability of pCer to displace cholesterol both at low and high cholesterol concentrations. Interestingly, an inverse cholesterol-mediated ceramide displacement from its tight packing with sphingomyelin is clearly observed. These observations in model membranes in the absence of the lipids commonly used to form a liquid-disordered (L_α) phase support the proposed role of raft-like domains (Silva et al, 2007) rather than ceramide, in regulating ceramide-induced platform formation within cell membranes.

* Busto J.V. et al. (2009) Coexistence of immiscible mixtures of palmitoylsphingomyelin and palmitoylceramide in monolayers and bilayers. *Biophys. J.*, in press.
* Silva L.C. et al. (2007) Ceramide-domain formation and collapse in lipid rafts: membrane reorganization by an apoptotic lipid. *Biophys. J.*, 92(2): 502-516.

2543-Pos

Material Properties of Lipid Membranes from Molecular Dynamics Simulations

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Experimental data for many important properties of lipid bilayers are scarce and uncertain. This includes for instance area per lipid, area and volume compressibilities, area expansion coefficients and heat capacity.

Present simulation techniques can often give such properties more easily and sometimes with better accuracy than experiments. Simulation results depend, however, upon potential parameters that yet are not enough tested and validated. Still, the time is getting ripe for systematic calculation of membrane properties using simulation techniques. This is useful both since it will provide better values for a number of membranes properties but also since a more serious test of the membrane force field parameters will push the refinement and development on this side.

Initially, the study has concentrated on area per lipid, area compressibility and bending modulus. We have shown that the inverse apparent area compressibility modulus obtained from the area fluctuations of the system shows a linear variation with system size. From this, the the true area compressibility modulus can be obtained by extrapolating to small areas. From the term that varies linearly with system size and is due to undulations, the bending modulus of the bilayer can easily be calculated. The method has been applied to several lipids including DPPC and DMPC.