

1062-Plat**Investigating the Nanodomain Structure of Sphingomyelin/Cholesterol Membranes Using 2H NMR**

Amirmohamad Keyvanloo, Martin Zuckermann, Jenifer Thewalt.
Simon Fraser University, Burnaby, BC, Canada.

We have used ^2H NMR to study the dependence of the phase and nanodomain structure of sphingomyelin/cholesterol membranes on sterol content and temperature. NMR spectra of N-palmitoyl (D31)-D-erythro-sphingosylphosphorylcholine (PSM) were taken for temperatures from 25 to 70°C and cholesterol concentrations 0 - 40%. Three distinct phases are observed: solid-ordered (so), liquid-disordered (ld) and liquid-ordered (lo). The constructed phase diagram exhibits both so+lo and ld + lo phase coexistence regions, however macroscopic (micron-sized) coexistence of ld + lo does not occur. Instead, we observed line-broadening in the ld + lo region which was characterized by examining the cholesterol dependence of the quadrupolar splittings and line-widths of the peaks in the depaked spectra, at a given temperature. These observations were analyzed in terms of fast exchange of lipids between ld and lo nanodomains. However, by analyzing the depaked spectrum, information from all orientations of the lipid long axis, which constitutes the powder pattern, is pooled. This may obscure the results used to characterize domain size. To attempt to gain new insight into the structural properties of the domains in PSM/cholesterol membranes we used selectively deuterated sphingomyelin, labeled at C9 of the N-linked palmitoyl chain, to measure the orientation dependence of T_2 relaxation. NMR measurement of T_2 relaxation time shows that it does depend on the orientation of the axis of symmetry of the motion - the lipid long axis - relative to the magnetic field. For example, for small angles T_2 is found to be as short as 200 μs whereas the 90 degree orientation has a relaxation time longer than 600 μs . We will present data on the orientation-specific influence of cholesterol on the sphingomyelin spectrum's relaxation time and discuss lo + ld nanodomain structure in this context.

1063-Plat**The Effect of Cholesterol on the Electroporation Process**

Iris van Uitert, Séverine Le Gac, Albert van den Berg.

University of Twente, Enschede, Netherlands.

The goal of this work is to better understand the pore formation mechanisms during electroporation, a technique that uses an electric field to transiently permeabilize membranes and introduce foreign material such as genes into cells. We investigate the contribution of different components found in cell membranes to this process by using planar models of cell membranes whose composition is easily varied, and focus here on cholesterol. Pore formation is studied by measuring the electroporation threshold (V_{th}), the potential at which pores appear in the membranes.

We start with simple mono-phospholipid membranes to which cholesterol is added. Cholesterol stabilizes DPhPC (1,2-diphytanoyl-sn-glycero-3-phosphocholine) membranes until 10% cholesterol (V_{th} of 255 mV vs. 200 mV without cholesterol) and subsequently strongly destabilizes it (100 mV at 35%). With L- α -PC (L- α -phosphatidylcholine) an opposite trend is observed: a slight destabilization below 20% cholesterol (V_{th} decrease from 165 to 110 mV) followed by a marked stabilization (420 mV at 50%). This concentration- and phospholipid-dependent effect is in good agreement with other reports and can be correlated to the molecular organization in the membrane and its thickness. However, cholesterol preferably interacts with saturated sphingolipids in natural membranes to form liquid-ordered microdomains. Sphingolipids are consequently taken into account, and modeled with sphingomyelin (SM). Sphingomyelin does not interact with L- α -PC, and strongly destabilizes L- α -PC membranes (V_{th} of 50 mV for 85% SM). Conversely, the addition of cholesterol totally changes the properties of membranes prepared from various L- α -PC:SM ratios: we observe a step-wise stabilization of the membrane that can be correlated with the presence of different phases (gel, liquid-ordered and liquid-disordered) in the membrane. We are currently investigating other ternary systems to better understand the combined role of these three constituents on the process of pore formation.

1064-Plat**Lipid Raft Composition Modulates Sphingomyelinase Activity and Ceramide-Induced Membrane Physical Alterations**

Liana C. Silva^{1,2}, Anthony H. Futerman³, Manuel Prieto¹.

¹CQFM, Instituto Superior Técnico, Lisbon, Portugal, ²iMed.UL, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal, ³Weizmann Institute of Science, Rehovot, Israel.

Lipid rafts and ceramide (Cer)-platforms are membrane domains that play an important role in several biological processes. Cer-platforms are commonly formed in the plasma membrane by the action of sphingomyelinase (SMase) upon hydro-

lysis of sphingomyelin (SM) within lipid rafts. The interplay between SMase activity, initial membrane properties (i.e., phase behaviour and lipid lateral organization) and lipid composition, amount of product (Cer) generated, and how it modulates membrane properties was studied using fluorescence methodologies in model membranes. The activity of SMase was evaluated by following the hydrolysis of radioactive SM. It was observed that: i) enzyme activity and extent of hydrolysis is strongly dependent on membrane physical properties but not on substrate content, being higher in raft-like mixtures, i.e., those mixtures with l_d - l_o (liquid disordered-liquid ordered) phase separation, and ii) Cer-induced alterations are also dependent on membrane composition, namely on cholesterol (Chol) content. In the lowest-Chol range, Cer segregates together with SM into small, ~8.5 nm, Cer/SM-gel domains. Upon increasing Chol, the ability of Cer to recruit SM and to form gel domains strongly decreases. In the high Chol range, a Chol-enriched/SM-depleted l_o phase predominates.

Together, these data suggest that in biological membranes Chol in particular, and raft domains in general, play an important role in the modulation of SMase activity and in the regulation of membrane physical properties by restraining Cer-induced alterations.

1065-Plat**Real Time Fluorescence Microscopy Observations of Dehydration-Induced Domain Formation in Raft Forming Multi Lamellar Lipid Stacks**

Lobat Tayebi, Sean F. Gilmore, Atul N. Parikh.

UC Davis, Department of Applied Science, Davis, CA, USA.

Domain formation in multi-component lipid mixtures is a well-known phenomenon understood in terms of equilibrium phase separation of lipid components into distinct co-existing phases. Such phase-separated equilibrium domain morphologies have been extensively studied in mono- and bimolecular lipid configurations (e.g., Langmuir monolayers, and giant unilamellar vesicles), and to a lesser extent in multilamellar lipid configurations consisting of discrete, low number of lamellae (less than ten). We have run experiments that reveal pattern formation in thick lipid stacks consisting of several thousand lamellae. We observe the appearance of domains when a lipid cake - pre-hydrated by a prolonged exposure to humid air - are left standing in laboratory air. The domain patterns develop in concert with the evaporation of water from a nominally wet pre-hydrated lipids consisting of putative raft forming mixtures of egg-sphingomyelin, Cholesterol, and DOPC. Our results suggest that the dehydration of water-saturated lipid stacks provides the driving force to induce the lateral phase separation of the lipid mixture into the so-called liquid-ordered (cholesterol and sphingomyelin-enriched) and liquid-disordered phases. Our experiments enable studies of the dynamics of the domain pattern formation and the interactions between domains (e.g., long-term fusion) at low hydrations. We use a combination of microscopy tools, including Atomic Force Microscopy, fluorescence confocal microscopy, and bright-field microscopy, to determine the influence of interaction between the line tension and key elastic properties of the lipid membrane on the characteristics of the domain patterns. The observed dehydration-induced phase separation may have important consequences for freeze-drying or desiccation of lipid mixtures and living cells.

1066-Plat**Cholesterol-Dependence of the Rupture Activation Energy in Phase-Segregated Multicomponent Lipid Bilayers**

Ruby May A. Sullan^{1,2}, James K. Li¹, Gilbert C. Walker¹, Shan Zou².

¹University of Toronto, Toronto, ON, Canada, ²Steele Institute for Molecular Sciences, National Research Council Canada, Ottawa, ON, Canada.

The role of cholesterol in rafts assembly has been demonstrated in both model membranes and living cells. Here, we used AFM-based force mapping to investigate the influence of different cholesterol levels (5-40%) on the rupture of phase-separated multicomponent lipid bilayers consisting of DOPC/sphingomyelin/cholesterol. We report breakthrough forces for the coexisting phases, liquid ordered domains (Lo) and fluid disordered phase (Ld), at various loading rates. Breakthrough forces for both Lo and Ld phases increase with higher loading rate and decrease with increasing cholesterol concentration, consistent with the role of cholesterol in the formation of liquid ordered state. The breakthrough activation energies (30-60 kJ/mol) were calculated following the model for rupture of molecular thin films and compare favourably with reported values for the diffusion of lipid molecules in phospholipid bilayers using other techniques. This work has demonstrated the effective use of AFM-based force mapping to study the influence of cholesterol concentration on the nanomechanical stability of the coexisting phases of model membranes, providing fundamental nanomechanical insights on the role of cholesterol in the formation and stability of sphingolipid/cholesterol-enriched domains or rafts.