The effect of unsaturations on sphingomyelin and ceramide-induced lateral domain segregation in lipid membranes.

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Abstract

Sphingomyelins (SM) are important phospholipids in the plasma membrane of most cells. Due to their mostly saturated nature (commonly 16:0, 18:0 or 24:0 N-linked acyl chains), they affect the lateral structure of membranes. These SM can interact with cholesterol (Chol) and ceramide (Cer) and give rise to the formation of highly ordered lipid domains, either fluid or gel[1]. However, SM containing 24:1 N-linked acyl chains are also common in most tissues and have been described as unable to form ordered domains in the presence of Chol, apparently because of their unsaturated chain. Thus, unsaturated SM could act as a natural tool for preventing lateral phase separation in cell membranes[2]. The aim of this study was to describe the effects of different combinations of C16:0 (palmitoyl) and C24:1 (nervonoyl) sphingolipids in model membranes composed of DOPC:SM:Chol (2:1:1) + 30 mol% Cer in order to recreate a "raft-like" environment in the presence of ceramide, where SM can be C24:1 SM (nSM), C16:0 SM (pSM) or an equimolecular mixture of both, and Cer can be C24:1 Cer (nCer), C16:0 Cer (pCer) or an equimolecular mixture of both.

Results

DOPC:pSM:Chol

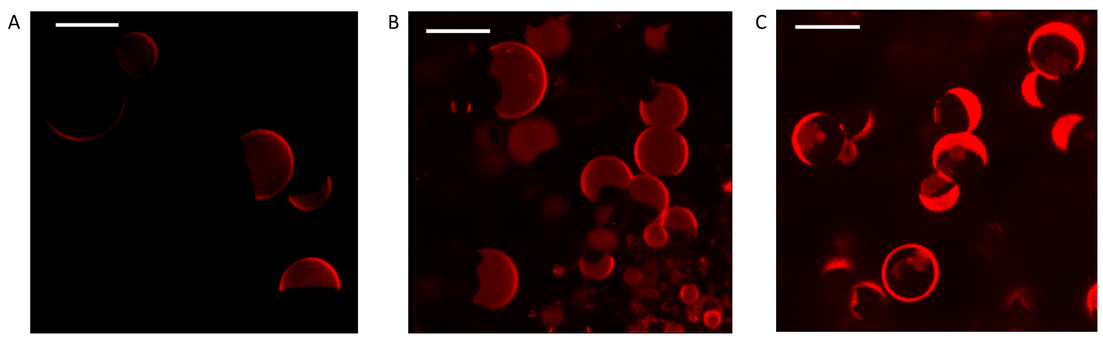

Figure 1: Confocal microscopy images of Liss-Rho-DOPE GUVs. Vesicle lipid composition was DOPC/pSM/CHOL (2:1:1, molar ratio) + 30% Cer, where Cer was 16:0 Cer [A], 24:1 Cer [B] or 16:0-24:1 Cer [C] . GUVs show heterogeneous distribution of the probe (L_β and L_d phases), where L_d phases are Rho-PE stained [A, B]. GUVs prepared with 16:0 and 24:1 Cer show three different phases [C]. Scale bar, 10 µm.

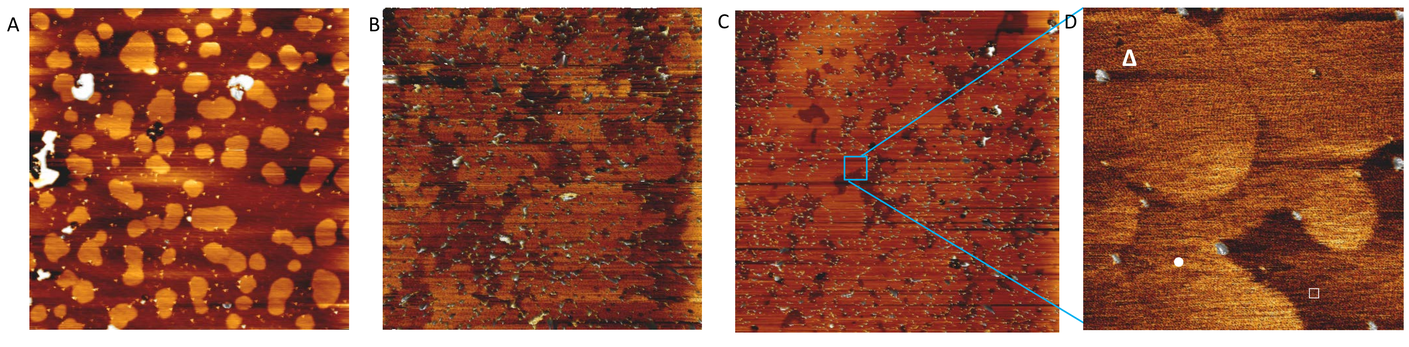

Figure 2: AFM images of supported planar bilayers (SPB). AFM topographic image of DOPC/pSM/CHOL (2:1:1, molar ratio) + 30% Cer, where Cer was 16:0 Cer [A], 24:1 Cer [B] or 16:0-24:1 Cer [C]. These images show coexistence of bright domains (SM/Cer) with a L_β structure in a darker(-fluid) DOPC enriched matrix [A,B]. However, [C] shows an additional segregated phase, more clearly seen in the magnification [D]. Scale: [A] 17x17 nm, [B] 24x24 nm, [C] 25x25 nm, [D] 2.1x2.1 nm. [D] Δ = L_β phase. ● = L_o phase. □ = L_d phase.

DOPC:nSM:Chol

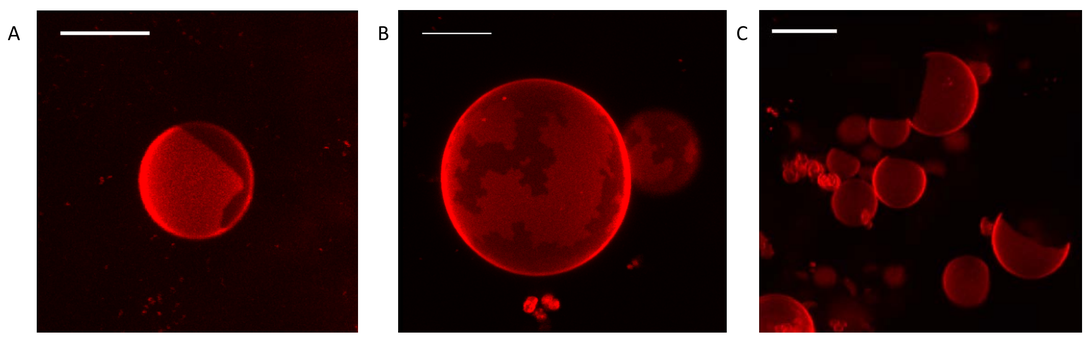

Figure 3: Confocal microscopy images of Liss-Rho-DOPE GUVs. Vesicle lipid composition was DOPC/nSM/CHOL (2:1:1, molar ratio) + 30% Cer, where Cer was 16:0 Cer [A], 24:1 Cer [B] or 16:0-24:1 Cer [C] . GUVs show the existence of two phases stained by Rho-PE with different intensity [A,B]. Only one phase is Liss-Rho-DOPE stained (L_d phase) [C]. Scale bar, 10 µm.

DOPC:pSM:nSM:Chol

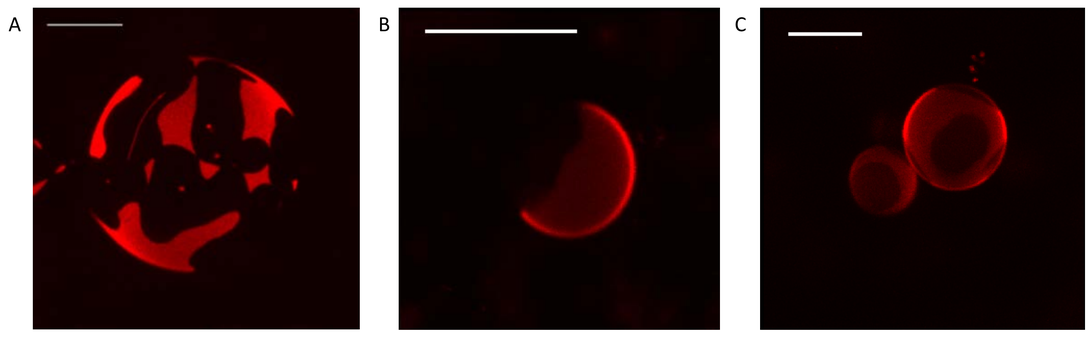

Figure 4: Confocal microscopy images of Liss-Rho-DOPE GUVs. Vesicle lipid composition was DOPC/pSM/nSM/CHOL (2:1:1, molar ratio) + 30% Cer, where Cer was 16:0 Cer [A], 24:1 Cer [B] or 16:0-24:1 Cer [C] . GUVs show the existence of two different phases (L_d stained by Liss-Rho-DOPE) [A,B]. The domain observed when both ceramides are present exhibits a partial partition of the fluorescent probe, that is not completely excluded [C]. Scale bar, 10 µm.

Force Spectroscopy

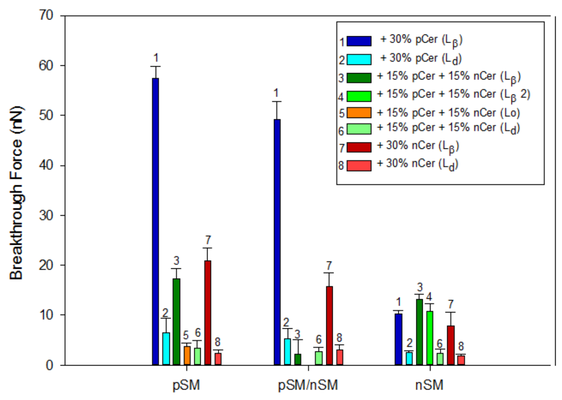

Figure 5: Breakthrough force (nN) of both gel phase (L_β), liquid ordered phase (L_o) and liquid phase (L_d) of the quaternary samples DOPC/SM/Chol/Cer.

Differential Scanning Calorimetry

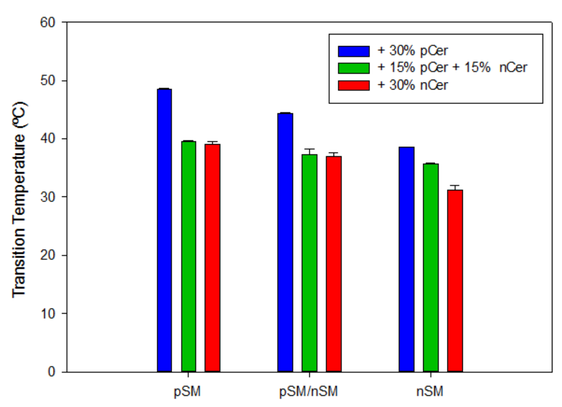

Figure 6: Transition temperature (T_m) of the quaternary samples DOPC/SM/Chol/Cer. Average ± SE are shown.

AFM images of supported lipid bilayers of DOPC/nSM/CHOL (2:1:1, molar ratio) + 30% Cer, showed that the presence of nSM instead of pSM caused a sharp reduction on bilayer stiffness measured for every phase. When both ceramides are present, two kinds of domains appear but these are separated from each other and both exhibit a higher nanomechanical resistance that single-Cer samples. AFM imaging of DOPC/pSM/nSM/Chol + 30% pCer reveals that there is only one single segregated gel phase for every case, even when both ceramides are present.

Conclusions

The exchange from pSM to nSM in quaternary samples of DOPC/SM/Chol + 30%Cer reduced the stiffness and T_m in all cases under study, while in ternary samples DOPC/SM/Chol it prevented phase separation[2]. In addition, nCer was shown to have a lower stiffening effect than pCer. When both pCer and nCer were present, segregated ceramide-enriched gel domains were formed. For pSM-containing samples two segregated gel phases with intermediate properties were present, while nSM-containing samples showed two segregated gel domains with different properties. These domains have a higher stiffness caused, probably, by some kind of inter-ceramide cooperation mediated by nSM. Finally, when both pSM and nSM are present, both ceramides tend to interact preferentially with pSM.

[1] Busto et al. (2014) Biophys. J. 106:621.
[2] Mate et al. (2014) Biophys. J. 106:2606.