Evidence of tryglyceride-phase incorporation into artificial bilayers for studying lipid droplets biogenesis

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Lipid Droplets (LD) are intracelular structures consisting on an apolar lipid core - composed mainly of triglycerides (TG) and steryl esters- which is surrounded by a phospholipid and protein monolayer. LDs originate in the ER bilayer, where TG synthesis concludes. The mechanisms underlying TG nucleation, size maduration and budding-off from the ER membrane are a matter of current investigations and the role of dewetting from cytosolic-bilayer interface appears to play a critical role. In order to contrast the nano-sized "blisters" of TG that some authors predict[1], here we formed free-standing bilayers by transferring films of a monolayer of mixed phosphatidylcholine(EPC)/TG in coexistence with TG microlenses (*i.e.* an excluded TG phase floating in the surface). These membranes were characterized by adding them the solvatochromic fluorescent probe Nile Red (NR) and observing them under spectral confocal microscope. Such bilayers exhibit fluorescence emission spectra comparable of bilayers of vesicles with similar composition (POPC and TO). By comparison with literature data and fluorescence spectra of EPC and TG monolayers, the peaks could be assigned to different phases, namely 1) PC membranes ($\lambda^{em}max=630\ nm$) bilayer and bilayer) and 2) TG isotropic phase ($\lambda^{em}max=570\ nm$). No microscopic structures could be observed at $\lambda^{em}max=570\ nm$. Diffusion of NR under this TG phase was characterized using FRAP analysis yielding values (D=2 $\mu m^2 s$) typical of model bilayer membranes, suggesting that the probe is diffusing in a 2D structure. This system appears appropiate for describing which is the distribution of the TG phase, that is, homogeneously among the intrabilayer space or in nanoscopic "blisters", by evaluating diffusion times obtained by FCS and FRAP.

References

1. Khandelia, H., et al., *Triglyceride blisters in lipid bilayers: implications for lipid droplet biogenesis and the mobile lipid signal in cancer cell membranes.* PLoS One, 2010. **5**(9): p. e12811.

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