Complex lamellar gel lipid phases enriched in ceramide and cholesterol: an AFM study in lipid membranes

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Abstract

Atomic force microscopy (AFM) has been applied to the characterization of palmitoylceramide (pCer) and cholesterol (Chol) incorporation into phospholipid-based supported planar bilayers (SPBs) at 22ºC. Phospholipids were dipalmitoyl phosphatidylcholine (DPPC) or palmitoyl sphingomyelin (pSM). Membranes of different compositions were prepared by the vesicle adsorption or the spin-coating procedures (strictly for pCer-containing mixtures) and analyzed with a combination of AFM imaging (domain segregation, bilayer thickness and roughness) and force spectroscopy (mechanical resistance to indentation).

The mixtures under study were pure phospholipids (pSM, DPPC), phospholipid:Chol (70:30), phospholipid:Chol:pCer (54:23:23) and phospholipid:pCer (90:10, 80:20 and 70:30). Binary phospholipid:pCer mixtures at increasing ceramide ratios gave rise to highly-resistant segregated domains with increasing extension but similar properties in terms of breakthrough forces, thicknesses and roughnesses. These ceramide-enriched domains are able to exclude a fluorescent lipid probe (DiIC18) due to their high intermolecular packing. Interestingly, these domains have been reported to disappear when model membranes become highly enriched in cholesterol in fluid membranes [1] or in the absence of a fluid phase [2] (our case).

Indeed, the ternary mixtures (54:23:23) gave rise to a homogenous lamellar gel phase with significantly different properties when compared to all of the other mixtures studied: ternary mixtures showed a reduced thickness and an intermediate roughness and mechanical resistance when compared to phospholipid:Chol (70:30) and phospholipid:pCer. These differences were statistically significant. More importantly, at those relatively high pCer and Chol concentrations in ternary mixtures, no mutual displacement of these molecules was observed, and these lipids establish a direct interaction between the amide group of ceramide and the hydroxyl group of cholesterol [3]. The data becomes relevant in the context of sphingolipid signaling and membrane platform formation.

References

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