Biophysical studies of cholesterol in unsaturated phospholipid model membranes

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Abstract:

Cellular membranes contain a staggering diversity of lipids. The lipids are heterogeneously distr ibuted to create regions, or domains, whose physical properties differ from the bulk membrane and play an essential role in modulating the function of resident proteins. Many basic questions pertaining to the formation of these lateral assemblies remain. T his research employs model membranes of well - defined composition to focus on the potential role of polyunsaturated fatty acids (PUFAs) and their interaction with cholesterol (chol) in

restructuring the membrane environment. Omega - 3 (n - 3) PUFAs are the main bioactive components of fish oil, whose consumption alleviates a variety of health problems by a molecular mechanism that is unclear. We hypothesize that the incorporation of PUFAs into membrane lipids and the effect they have on molecular organization may be, in part, responsible. Chol is a major constituent in the plasma membrane of mammals. It determines the arrangement and collective properties of neighboring lipids, driving the formation of domains via differential affinity for different lipids . T he m olecular organization of 1 -[2 H 31] palmitoyl -2- eicosapentaenoylphosphatidylcholine (PEPC - d 31) and 1 -[2 H 31]palmitoyl -2- docosahexaenoylphosphatidylcholine (PDPC -d 31) in membran es with sphingomyelin (SM) and chol (1:1:1 mol) was compared by solid - state 2 H NMR spectroscopy. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are the two major n - 3 PUFAs found in fish oil, while PEPC -d 31 and PDPC -d 31 are phospholipids containing the respective PUFAs at the sn - 2 position and a perdeuterated palmitic acid a t the sn - 1 position . Analysis of s pectra recorded as a function of temperature indicate s that in both cases, formation of PUFA - rich (less ordered) and SM - rich (more ordered) domains occurred. A surprisingly substantial proportion of PUFA was found to infil trate the more ordered domain. There was almost twice as much DHA (65%) as EPA (30%) . The implication is that n - 3 PUFA s can incorporate into lipid rafts, which are domains enriched in SM and chol in the plasma membrane, and potentially disrupt the activity of signaling proteins that reside therein. DHA, furthermore, may be the more potent component of fish oil. PUFA - chol interactions were also examined through affinity measurements. A novel method utilizing electron paramagnetic resonance (EPR) was develope d, to monitor the partitioning of a spin - labeled analog of chol , 3β - doxyl - 5α - cholestane (chlstn), between large unilamellar vesicles (LUVs) and met hyl - β - cyclodextrin (mβCD). The EPR spectra for chlstn in the two environments are distinguishable due to the substantial differences in tumbling rates , allowing the population distribution ratio to be determined by spectral simulation. Advantages of this approach include speed of implementation and a vo idance of potential artifact s associated with physical separation of LUV and mβCD . Additionally, in a check of the method, t he relative partition coefficients between lipids measured for the spin label analog agree with values obtained for chol by isothermal titration calorimetry (ITC). Results from LUV with different composition confirmed a hierarchy of decreased sterol affinity for phospholipids with increasing acyl chain unsaturation , PDPC possessing half the affinity of the corresponding monounsaturated phospholipid. Taken together, the results of these studies on model membranes demonstrate the potential for PUFA - driven alteration of the architecture of biomembranes, a mechanism through which human health may be impacted.

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