Solid State $^2$H NMR Studies of the Disordering of Raft-like Domains by N-3 PUFA

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Introduction

Research continues to examine the health benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA) found in fish oils. The major bioactive components are eicosapentaenoic acid (EPA, 20:5), with 20 carbons and 5 double bonds, and docosahexaenoic acid (DHA, 22:6), with 22 carbons and 6 double bonds. However, their molecular modes of action remain unclear. A suggested hypothesis is that these fatty acids are incorporated into membrane phospholipids and modify the structure of lipid rafts, thus affecting cell signaling.

We used solid-state $^2$H NMR spectroscopy to compare molecular organization of POPC-$d_{18}$ and SM-$d_{18}$ in POPC/SM/Cholesterol (1:1:1 mol) mixtures. For each phospholipid the palmitoyl chains (at sn-1 position for POPC and amide linked for SM) were perdeuterated. This system will serve as a control for studies of the effect of DHA and EPA on lipid raft-like domains.

$^2$H NMR Spectroscopy

NMR observes the interaction of nuclear spins (as spectra) with an applied magnetic field. The quadrupolar moment of $^2$H also interacts with the electric field gradient of the carbon-deuterium bond, which results in a broad spectrum. Angular motion results in averaging and thus narrowing of the observed spectrum, which is quantified as an order parameter ($S_{CD}$).

We observe a superposition of spectra for each $^2$H in a perdeuterated acyl chain (see sample spectra). By integrating the measured spectrum against the frequency, we calculate the first moment. This quantity is related to the averaged order parameter.

Sample Spectra

Sample spectra for PC/SM (1:1) and PC/SM/cholesterol (1:1:1) at mixtures at 30 °C. Terminal methyl group spectral components of separate domains are indicated with small circles.

Measured Moments

Plots show the temperature dependence of the moments of SM (reds) and POPC (blues) in the mixed (1:1, solid) and with cholesterol (1:1:1, dashed) membranes. The insets reveal the effect of mixing SM and POPC and the addition of cholesterol at 40 °C.

Discussion

The sample spectra presented reveal that DHA has greater integration in the more ordered SM-rich domains than EPA. This suggests that DHA may be the more biologically active component in fish oils. Now what happens to the SM domains? To establish a baseline we have first looked at POPC/SM/Cholesterol mixtures.

Homogeneous Mixing

No Mixing (Domains)

SM Mixing

POPC Mixing

The moments for POPC-$d_{18}$ and SM-$d_{18}$ in the POPC/SM mixtures (with and without cholesterol) do not match, indicating domain formation. Also the molecular organization for SM is significantly altered unlike POPC in the mixture, suggesting significant mixing of POPC in SM domains and less mixing of SM in POPC domains. Using a lateral diffusion model (with fast exchange) we find the lifetime for residency in domains to be less than 17.1 μs, corresponding to a size less than 1000 nm$^2$ (180 lipids). The equal effect of cholesterol on POPC and SM order, suggests it may not have a preference for either domain. Future work will look at the effect of DHA and EPA on SM organization.

References
