PFG NMR Visualization of the Exchange of Lipids between Nanodomains and their Surroundings in Model Lipid Membranes

M. Sanders (UF, Chemical Engineering); S. Vasenkov (UF, Chemical Engineering)

Introduction

Eukaryotic cell membranes are believed to be heterogeneous in composition over length scales as small as 100 nm. Observation and characterization of domain formation requires experimental techniques that are capable of obtaining data with sufficient spatial resolution in a non-invasive manner. We use high field (17.6 T) pulsed field gradient NMR with high gradient strengths (up to 30 T/m) to measure the lateral diffusion of lipids in model membranes. Ternary mixtures of phospholipids, sphingolipids, and cholesterol are known to be a reasonable model for the composition of the cell membrane. Recent literature suggests evidence of the formation of nanodomains in model membranes of certain compositions at temperatures near the melting transition temperature. It is the intention of this work to gain more insight into lipid dynamics in and around these nanodomains. Only then is it possible to fully understand the structure-dynamic relationship in domain-forming lipid membranes.

Experimental

A ternary mixture of DOPC/DPPC (1:1) with 35% cholesterol is used to make planar-supported multibilayer stacks. The preparation protocol involves dissolving the lipids in solvent, depositing the ternary mixture on glass plates (6*7 mm²), evaporating off the solvent, and hydrating the lipids to promote self-orientation into bilayers. Stacks of 30-35 plates are then oriented at the magic angle using specially-made inserts designed for standard 10 mm NMR tubes. The measurements of lipid self-diffusion in bilayers were carried out on a wide-bore 17.6 T spectrometer located at the AMRIS facility. For most diffusion measurements, the standard stimulated echo PFG NMR sequence was used. The high performance characteristics of our spectrometer allows us to carry out diffusion measurements in oriented multibilayer stacks over a wide range of diffusion times ($\Delta$ = 10 – 240 ms) with sufficient S/N. PFG NMR signal attenuation was measured as a function of gradient strength. The lateral diffusivities were obtained from one-exponential or two-exponential fits of the attenuation curves.

Results and Discussion

Figure 1 shows an example of the measured PFG NMR attenuation curves in lipid bilayers containing lipid nanodomains. Each curve corresponds to measurements using different diffusion time and the corresponding root mean square displacement. The solid lines show biexponential fits of each set of data yielding diffusivities and ensemble fractions for lipids diffusing inside nanodomains and those diffusing in the membrane areas surrounding the domains. It is seen in the figure that there is a distinct dependence of dynamic behavior on diffusion time. Analysis of this dependence suggests evidence for exchange between the two ensembles of lipids. The extent of this exchange is further seen to be a function of temperature.

Conclusions

The correlation between exchange and temperature suggests a dependence of characteristic domain size on temperature. Results of this work also provide further evidence of the formation of nanodomains at temperatures relatively near the transition temperature. Further studies of exchange and restricted-diffusion inside the domains are expected to yield valuable information about the permeability of domain boundaries.

Published Work