Observation of Time-dependent Diffusion Behavior of Lipids in Model Lipid Membranes Using Pulsed Field Gradient NMR with High Gradient Strength

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Introduction
Lipid rafts are small, functional domains which are expected to exist in cell membranes and are implicated in many cellular processes such as signal transduction. The study of lipid lateral diffusion in the presence of these domains could lead to a better understanding of lipid raft properties as well as their functions in the cell. These studies require the use of experimental techniques that are capable of measuring data with sufficient spatial resolution in a non-invasive manner. We use pulsed field gradient NMR with high gradient strengths (up to 30 T/m) to measure the lateral diffusion of lipids in model membranes which are known to be reasonable models for the composition of the cell membrane. Recent literature suggests evidence of the formation of domains in model membranes under certain conditions (i.e. certain compositions and temperatures near the melting transition temperature) which are comparable in size to lipid rafts in the cell. It is the intention of this work to gain more insight into lipid dynamics in and around these small domains and use these studies to provide new information about fundamental properties of the system.

Experimental
A ternary mixture of DOPC/DPPC (1:1) with 35% cholesterol is used to form planar-supported multibilayer stacks. The preparation protocol involves dissolving the lipids in solvent, depositing the ternary mixture on thin 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, which were developed in our group for standard 10 mm NMR tubes. The measurements of lipid self-diffusion in bilayers were carried out on a wide-bore 750 MHz NMR spectrometer available at the AMRIS facility of the University of Florida. The high performance characteristics of our spectrometer affords the use of small diffusion times (≥ 8 ms) under the conditions of the narrow-pulse approximation which allows for distortion-free monitoring of time-dependent and displacement-dependent diffusion behavior of lipids with superior signal-to-noise. For most diffusion measurements, the standard stimulated echo PFG NMR sequence was used to measure signal attenuation as a function of gradient strength. The lateral diffusivities were obtained from one-exponential or two-exponential fits of the resulting attenuation curves.

Results and Discussion
Fig. 1 shows examples of diffusion coefficients (right axis, striped bars) and phase fractions (left axis, solid bars) obtained from PFG NMR diffusion measurements conducted at two diffusion times, 14 ms and 179 ms, at a temperature very near the transition temperature for this mixture. Similar results were obtained for a wide range of temperatures. In Fig. 1, the domain phase fraction is reduced as diffusion time is increased indicating that the ensemble of molecules that start and end their trajectories inside one and the same domain decreases with increasing time. It was concluded that this time-dependent behavior was a consequence of molecular exchange of lipids between the domain and non-domain phases during the diffusion time. NMR relaxation effects were shown to have a negligible effect on the results at the temperatures of interest (i.e. near the transition temperature).

Conclusions
This is the first observation of time-dependent diffusion behavior for lipid membranes of any composition by high gradient PFG NMR. These PFG NMR diffusion results were used in combination with dynamic Monte Carlo simulations to obtain first estimates of the permeability of domain boundaries at temperatures near the transition temperature.

References