Lateral Diffusion in Lipid Membranes on Nanoscale by High Gradient PFG NMR

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Introduction

It has been suggested that cell membranes are heterogeneous in composition over length scales as small as 100 nm. In particular, domains known as lipid rafts are believed to exist in cell membranes and participate in may membrane functions. This area of research has received increasing attention of the scientific community over the past decade. However, because of the small size of rafts (~100 nm), there is still insufficient insight into how and why lipid rafts form and function in cells. This work demonstrates the feasibility of non-invasive studies of lipid self-diffusion in membranes on the nanoscale using ^{1}H PFG NMR spectroscopy with high (up to 35 T/m) gradient amplitudes. Application of high gradients affords for the use of sufficiently small diffusion times under the conditions of the narrow pulse approximation. As a result, PFG NMR studies of partially restricted or anomalous diffusion in lipid bilayers become possible over length scales as small as 100 nm.

Experimental

Sample Preparation: A ternary mixture is of DOPC/Sphingomyelin/cholesterol combined with naphthalene is deposited on 6*7mm glass plates. The lipids are hydrated with D_2O to allow for self-orientation of the lipids into bilayers. 35-40 plates are stacked and the multibilayer stacks are allowed to equilibrate[1]. The hydrated stacks are then placed into specially-made inserts, which orient the bilayers at the magic angle (54.7°with respect to B_o) and fit in standard 10 mm NMR sample tubes. Diffusion Measurements: Diffusion experiments were conducted using the 17.6 T spectrometer located at the AMRIS facility on the University of Florida campus. The ^{1}H NMR diffusion experiment employed a stimulated echo PFG NMR pulse sequence. Lateral diffusivities were determined from one- or two-exponential fit of the PFG NMR attenuation curves.

Results and Discussion

Figure 1 shows dependence of lateral diffusivity of lipids in the 3-component lipid membranes on inverse temperature. Results obtained at AMRIS (750 MHz) are plotted together with the PFG NMR data measured at lower B_0 field[2]. Below the transition temperature for the studied lipid membrane (T_m=25°C), two diffusivities are recorded corresponding to the self-diffusion of two different ensembles: lipids diffusing in a liquid-disordered continuous phase and lipids diffusing in liquid-ordered domains with sizes in the range of 1 micron. Above the T_m, there is a single diffusivity for lipids diffusing in a domain-free membrane. Good agreement between the data at different B_0 strength rules out an occurrence of susceptibility artifacts under our measurement conditions. The PFG NMR data agree well with the corresponding results obtained by fluorescence correlation spectroscopy for larger lipid dispalcements[3].

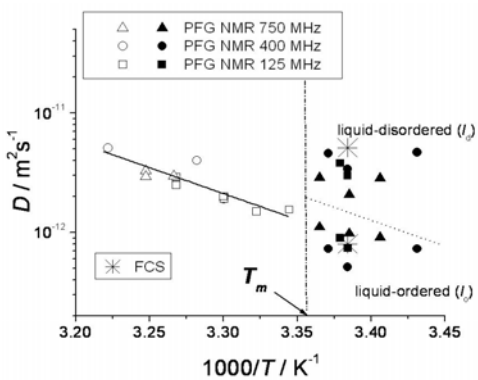

Figure 1: Lipid Diffusivity vs. Inverse Temperature

Conclusions

This work demonstrates that it is feasible to adapt high gradient PFG NMR for studies of lateral diffusion in lipid membranes on the nanoscale.

References

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[2] Ulrich, K., *et al*., Magnetic Resonance Imaging, **25**, 493-496 (2007); Ulrich, K., *et al*., Langmuir, submitted (2008);
[3] Kahya, N., *et al*., Journal of Biological Chemistry, **78**, 28109-28115 (2003).