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Influence of Domains on Lateral Diffusion in Lipid Bilayers: PFG NMR Study

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1. Introduction

Recent experimental studies have revealed the existence of microdomains in cell membranes [1]. These microdomains or "lipid rafts" are characterized by an enhanced concentration of cholesterol and sphingomyelin. Lipid rafts are believed to play an important role in many functions of biomembranes, such as signal transduction. Similar microdomains have also been found in multicomponent lipid bilayers [2], which are considered to be good model systems for mammalian cell membranes. The present work focuses on the influence of domains in model lipid membranes on the lipid self-diffusion. A meaningful study of this influence requires an ability of monitoring lipid diffusion in the broad range of displacements covering both the displacements, which are smaller and larger than the domain size. As a result the information on: (i) the diffusivities in and outside of domains, i.e. in the liquid-ordered (l_o) and in the liquid-disordered (l_d) phases and (ii) the possible transport barriers on the domain margins can be directly obtained.

2. Experimental

3-Component oriented multibilayer stacks on solid support (1,2-dioleoyl-sn-glycero-3-phosphocholine/cholesterol/spingomyelin with a molar ratio of 1:1:1) were used as a model system. The degree of orientation was determined by ^{31}P and ^{1}H NMR spectroscopic studies using goniometer probe. The measurements of self-diffusion in lipid bilayers were carried out by using pulsed field gradient (PFG) NMR with ultra-high magnetic field gradients. Application of this method for studies of diffusion in lipid membranes presents an attractive alternative to the well-established optical techniques such as fluorescence correlation spectroscopy and single particle tracking using optical or fluorescence microscopy. In comparison to the latter techniques, the application of PFG NMR does not require any perturbing labelling of diffusing species. It also allows collecting diffusion data from all the molecules in membranes under study, which have a selected type of nuclei with a non-zero gyromagnetic ratio. In contrast to the previous PFG NMR diffusion studies of lipid multibilayers [3], in the present work the PFG NMR technique with ultra-high magnetic field gradients (up to 35 T/m) has been used [4]. An application of this novel technique allows carrying out diffusion studies for very small diffusion times and for molecular root mean square displacements as small as ~100 nm.

3. Results

Fig. 1 shows examples of the measured ^{1}H PFG NMR attenuation curves. The measurements have been performed by using the stimulated echo sequence, if not

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indicated otherwise. Fitting of the attenuation curves has shown that for the temperatures lower or equal to 24 °C the two-exponential fit, which implies the existence of two diffusivities, produces better results than the one-exponential fit. At the same time, for the temperatures higher than 24 °C it has been sufficient to use single-exponential curves (corresponding to a single diffusivity) to fit the experimental data satisfactorily.

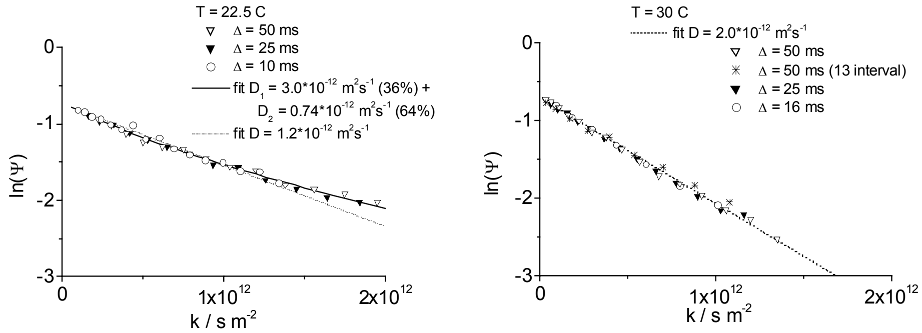

Fig. 1: ^{1}H PFG NMR attenuation curves measured for two different temperatures. The figure on the left (right) side shows the data points including a two-exponential (one-exponential) fit.

The data analysis has been performed in a similar manner as that used for the interpretation of diffusion data recorded in inhomogeneous nanoporous solids [5]. The diffusivities obtained by using the two-exponential fit were assigned to those in the liquid-ordered (l_o) and in the liquid-disordered (l_d) phases of the lipid bilayers. In general, the obtained diffusivities were found to be in good agreement with the previously reported data [2, 3] for the same system and for displacements, which were larger than the smallest displacements recorded in our studies.

4. Conclusion

Our first results show great potentials of the novel PFG NMR technique with ultra-high magnetic field gradients for diffusion studies in multibilayer stacks on solid supports.

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