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Anisotropic Water Diffusion in Macroscopically Oriented Lipid Bilayers Studied by Pulsed Magnetic Field Gradient NMR

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The anisotropy, $D_{//}/D_{\perp}$, of water diffusion in fully hydrated bilayers of dimyristoylphosphatidylcholine at 29°C has been measured by pulsed magnetic field gradient (pfg) NMR. By using NMR imaging hardware to produce magnetic field gradients in an arbitrary direction with respect to a stack of macroscopically aligned lipid bilayers, translational diffusion of water was measured as a function of the angle between the direction of the magnetic field gradient and the normal of the lipid membrane. The observed diffusion coefficient is found to depend strongly on this angle. The anisotropy cannot be accurately determined due to the very small value of $D_{\perp}$, but a lower limit of about 70 can be estimated from the observed diffusion coefficients. The results are discussed in terms of the relatively low permeability of water across the lipid bilayer, instrumental limitations, and/or possible defects in the lamellae.

INTRODUCTION

Pulsed field gradient (pfg) NMR spectroscopy has been used extensively for diffusion measurements for many years (*1, 2*). This technique provides one of the most attractive methods for investigations of molecular transport in biological cells, lipid membranes, and lyotropic liquid crystals, as no perturbing labeling of the molecules under study is necessary. While quite straightforward in isotropic systems, the method generally suffers from dipolar dephasing in anisotropic systems. For small molecules in solution the method can still be used to probe anisotropic diffusion (*3–5*), but for molecules with large dipole couplings, such as water and amphiphilic molecules in lamellar liquid crystalline phases, special methods to remove the dipole interaction become necessary. Though recent methods involving heteronuclear (*6*) or homonuclear (*7, 8*) dipolar decoupling have proven successful, the main method to determine the translational diffusion of lipids and water in lamellar liquid crystalline phases involves macroscopically aligned systems. For a recent review on the topic see Lindblom and Orädd (*2*). In this method the dipolar coupling is removed by orienting the bilayer normal at an angle of 54.7° with respect to the main magnetic field (*2*). In most probe designs, the magnetic field gradient is directed parallel to the main field and the measured diffusion is therefore a combination of motion perpendicular ($D_{\perp}$) and parallel ($D_{//}$) to the lipid bilayer. In such studies it is assumed that $D_{\perp}$ is negligible in comparison to $D_{//}$ and the lipid lateral diffusion coefficient, D_{L}, is directly obtained after a geometric correction. While this assumption certainly is valid for the lipid diffusion in a bilayer, where lipid exchange between different bilayers is very slow, it might not be so in measurements of the water diffusion in a lamellar phase. Water permeates the lipid bilayers fairly easy and a much smaller anisotropy ($D_{//}/D_{\perp}$) for the water diffusion is expected. In order to measure $D_{//}$ and $D_{\perp}$ we have used an NMR imaging microscopy system to create the pulsed magnetic field gradients allowing for the gradient vector to be set at an arbitrary angle relative to the oriented lipid membrane. Thus, the translational diffusion coefficient of water of a fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayer has been determined, for the first time, as a function of the angle between the gradient and the membrane.

EXPERIMENTAL

Sample preparation. Dry powder of DMPC (Sigma Chemical Co., St. Louis, MO) was dissolved in a mixture of 1-propanol and methanol (Merck, Darmstadt, Germany) in the proportion 4 : 1, to a concentration of 10 mg powder/mL. A total of 30 μl of the solution was added to each of 55 glass plates (14 × 4.7 mm) and the solvent was evaporated in air and then put in low vacuum over night. This procedure resulted in a homogeneous film covering the glass plates. The glass plates were then stacked in a sample tube with quadratic cross-sectional area. The sample tube was sealed in one end and put in an Eppendorff tube with a small amount of water at the bottom and the sample was allowed to equilibrate in this humid atmosphere at 30 to 40°C for about one week. During that period, the sample became transparent as the fully hydrated lamellar liquid crystalline phase was formed. After equilibration, an extra 5 μl of water was added and the other end of the sample tube was sealed. Before the NMR measurements, the sample was stored for another 2 days and the orientation was examined between crossed polarizers. At a glancing angle along the normal to the glass plates little

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birefringence was observed while small changes in the glancing angle gave considerable birefringence. This indicates that the membranes were well oriented having the normal coinciding with the normal of the glass plates. This was also verified by phosphorous NMR where a single sharp line with only minor traces of signal from unoriented sample was observed.

NMR experiments. The diffusion experiments were performed at 29°C on a Chemagnetics Infinity 400 MHz spectrometer with micro-imaging equipment capable of producing magnetic field gradients in the x-, y-, and z-directions by means of a shielded gradient stack (Resonance Research Inc.) driven by three Techron 7781 gradient amplifiers. Note that the applied magnetic field, B_0, is always along the z-direction, while the change in the field can be x-, y-, or z-dependent; e.g., the gradient strength in the x-direction is determined by dB_z/dx. The temperature of the sample was regulated by a heated air stream passing the sample and the water cooling of the gradient stack was regulated by a Neslab CFT-75 to produce the same temperature in the stack. The sample was placed horizontally into a home-made goniometer insert fit into the 20-mm RF coil of a 400-MHz ^{1}H-probe for microimaging (Fraunhofer Institute of Biomedical Engineering) and the orientation of the sample was adjusted by the goniometer until the sample was oriented with the bilayer normal at the magic angle (54.7°) with respect to the main magnetic field. The optimum orientation was determined by observing the spectral lineshape and the peak amplitude of the water signal, while the sample orientation was adjusted in small rotational steps. When the angle between the normal to the glass plates and the main magnetic field (ϕ) was close to the magic angle a significant narrowing of the water signal was observed as a result of the canceling of the dipolar broadening (*2*). This narrowing was very sensitive to ϕ and a deviation of about 0.5° from the magic angle resulted in a loss of more than 90% of the water peak amplitude in a spin-echo experiment. With the sample properly oriented it was possible to perform pfg NMR diffusion measurements in the same way as for isotropic solutions (*2*).

The water diffusion coefficient was measured with a *l*ongitudinal *e*ddy-current *d*elay (LED) pfg experiment (*9*) especially designed for this hardware. In each diffusion experiment the gradient amplitude, g, was varied between 0.02 and 0.90 T/m while keeping the rest of the variables constant. The gradient duration time, δ, and the g range were adjusted to get signal decays of about two orders of magnitude in all experiments, and two dummy gradients were applied prior to each measurement. The eddy-current delay was set to 50 ms to prevent signal artifacts from the slowly decaying residual gradients (*9*). The 90° pulse width was 46 μs and the waiting time between consecutive scans was more than $3T_1$, the longitudinal relaxation time. By an appropriate combination of the x-, y-, and z-gradients it was possible to direct the field gradient vector at an arbitrary angle with respect to B_0 or the bilayer normal. The gradient strength was determined by measuring the known diffusion coefficient of H_2O of a sample of water doped with 10 mM Cu^{2+} ions to enhance the T_1-relaxation and thus decrease the experimental time. The water diffusion at 29°C was taken to be $2.54 * 10^{-9}$ m^2/s (*10*). The diffusion time in this experiment was 257 ms and δ was 1 ms, while the experiments on the oriented bilayer samples were performed with a diffusion time of 107 ms and a δ between 1–11 ms.

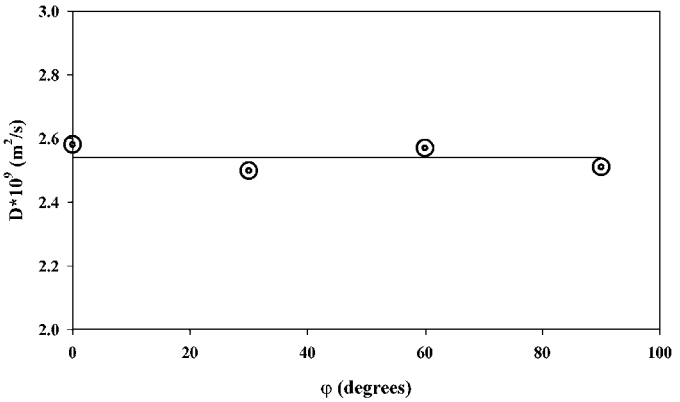

FIG. 1. Water diffusion measured as a function of φ for a sample with neat H_2O at 29°C. The horizontal line represents the water diffusion coefficient at 29°C according to Mills (*10*).

RESULTS

Figure 1 shows the results of varying the angle, φ, between the gradient direction and B_0 in a sample of plain water. The observed self-diffusion coefficient, D, does not depend on the direction of the gradient vector, reflecting the isotropic diffusion of water in this sample.

Anisotropic water diffusion was observed in the macroscopically aligned lamellar sample (Fig. 2). The gradient direction,

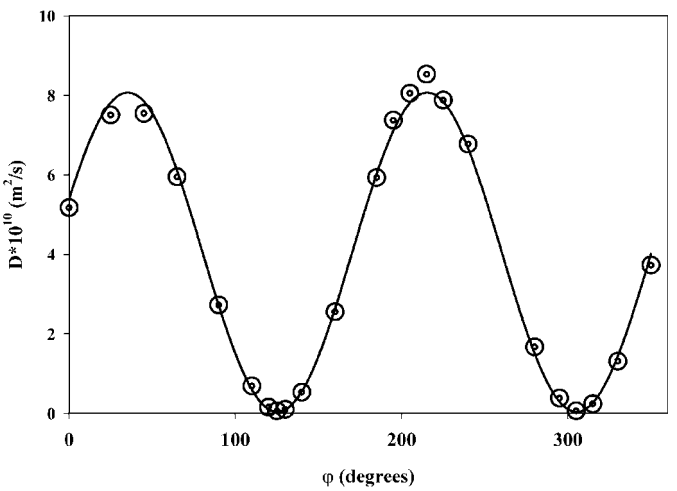

FIG. 2. Water diffusion in the oriented sample of DMPC/H_2O measured as a function of φ at 29°C. The solid line represents the best fit to Eq. [1].

φ, was varied while holding the sample orientation at the magic angle with respect to B_0. The separation of the water diffusion into $D_{//}$ and $D_\perp$ gives the following expression of the observed diffusion coefficient (*2*),

$$D_{obs} = D_{//} \sin^2(\varphi + \phi) + D_\perp \cos^2(\varphi + \phi). \qquad [1]$$

In this expression ϕ represents the angle between the bilayer normal and B_0 and is therefore set to the magic angle, 54.7°. The line in Fig. 2 shows the best fit to Eq. [1]. The diffusion coefficients obtained from the fit are $D_{//} = (8.01 \pm 0.07) * 10^{-10}$ m^2/s and $D_\perp = (0.04 \pm 0.07) * 10^{-10}$ m^2/s.

DISCUSSION

A comparison of the diffusion coefficients obtained in this work with previous investigations, performed with NMR techniques on both macroscopically aligned and nonoriented lamellar liquid crystalline phases(*11–13*), shows that the values for $D_{//}$ generally are in the order of (8-17) $* 10^{-10}$ m^2/s; i.e., they compare reasonably well with our results. However, $D_\perp$ obtained in this work is considerably smaller than that obtained previously by Callaghan and co-workers (*11*). The discrepancy might be an effect of the difference in the preparation of the oriented bilayers. In this work much effort was put in getting samples with as few defects of any kind as possible. Note that restricted diffusion caused by the glass plates in the stack is ruled out, since the distance between the glass plates is approximately 10 μm, which is large compared to the root mean square displacement $\sqrt{6D\Delta}$ for perpendicular diffusion.

The perpendicular diffusion coefficient can be estimated from the equation derived by Tanner for the long-time diffusion coefficient in a stack of equally spaced bilayers (*14*)

$$D_{lr} = \frac{\kappa l}{\left(\frac{\kappa l}{D} + 1\right)}, \qquad [2]$$

where D_{lr} is the effective self-diffusion coefficient observed for long times, D is the true microscopic self-diffusion coefficient for short observation times, l is the bilayer spacing, and κ is the bilayer permeability. Using $l = 100$ Å, $\kappa = 5 * 10^{-6}$ m/s (*15*) and $D = 10^{-10}$ m^2/s the effective self-diffusion coefficient is calculated to be $5 * 10^{-14}$ m^2/s. This value is below the limit of what is possible to measure with our equipment. Therefore, it is no surprise that the error in $D_\perp$ obtained from the fit is of the same order of magnitude as the determined value. It should be noted, however, that there is also a possibility that the actual value of $D_\perp$ is larger than the value obtained from Eq. [2], since the oriented membrane system may contain defects of different kinds, like cracks and dislocations in the bilayer stacks. Such defects would give water molecules many different pathways of travel, resulting in a larger observed diffusion coefficient than that determined by permeability only.

The magnitude of the water diffusion anisotropy cannot be accurately determined in this study since the experimental hardware is incapable of measuring such small $D_\perp$-values. It is, however, possible to obtain a lower limit of the anisotropy, calculated to be $(8.01 - 0.07)/(0.04 + 0.07) = 72$. In an ordinary pfg experiment, where the angle between the field gradient vector and the bilayer normal is 54.7°, it can then be calculated, using this value of the anisotropy, that the observed diffusion coefficient differs from $D_{//}$ by only about 1%. Thus, it is highly justified to disregard the diffusion motion perpendicular to the bilayers in the determination of water diffusion coefficients with pfg NMR on oriented membranes.

Further investigations are in progress on systems, in which the water permeability is enhanced by additives and/or by the formation of porous membranes. In those systems it is expected that the perpendicular motion will make a larger contribution to the observed diffusion coefficient.

To summarize, we have shown that microimaging equipment can easily be modified to include a pfg NMR diffusion facility with the ability to set the magnetic field gradient at an arbitrary angle with respect to a macroscopically aligned lipid membrane oriented at the magic angle in the B_0-field. The great advantage of the NMR microimaging instrument, as shown in this work, is that it enables measurements of anisotropic diffusion.

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