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Pulsed Field Gradient-Spin Echo NMR Studies of Water Diffusion in a Phospholipid Model Membrane

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ABSTRACT Water diffusion in the lamellar phase of egg phosphatidylcholine (egg PC)-water was studied by ^{1}H NMR using the pulsed field gradient-spin echo method. The curvature of diffusion plots obtained with egg PC-water mixtures indicates that water diffusion is highly anisotropic with respect to lipid lamellae. This was confirmed by measurements made on macroscopically aligned egg PC-water as a function of orientation that categorically establish $D_{\parallel}/D_{\perp} \gg 1$, where the respective subscripts refer to parallel and perpendicular to the lipid bilayer. A smooth, monotonic dependence on water concentration was observed for water diffusion in aligned egg PC-water, varying at 25°C from $D_{\parallel} = 1.2 \times 10^{-10}$ m^2 s^{-1} at $n = 4.9$ mol water/lipid to $D_{\parallel} = 4.0 \times 10^{-10}$ m^2 s^{-1} at $n = 18.6$ mol water/lipid. The diffusion is approximately a factor of 10 slower than in pure water because of water binding and restriction to translational motion within the aqueous layer. No evidence for a sudden drop in water diffusion coefficient at a specific water content, as previously reported with egg PC-water mixtures (Lange and Gary Bobo. 1974. *J. Gen. Physiol* 63:690–706), was detected. A morphological reorganization of lamellar domains, which in random orientational distribution comprise lipid-water mixtures, is the likely explanation. The study of aligned lipid-water systems is manifestly preferable.

INTRODUCTION

Water plays a fundamental role in determining the structure of membranes and is intimately involved in membrane function. Its presence is intrinsic to the formation of the basic phospholipid bilayer, and the degree of hydration is a determinant of headgroup and acyl chain motions (Ulrich and Watts, 1994; Ho et al., 1995; Holte and Gawrisch, 1996). Interactions with membranes, moreover, modify the behavior of water. The concept that the reorientational mobility of water molecules is reduced in the vicinity of phospholipid headgroups is well established, whereas characterization of the restriction to translational motion remains poorly defined (König et al., 1994; Volke et al., 1994). The latter issue is addressed in the current study, which uses the ^{1}H-NMR pulsed field gradient-spin echo (PFGSE) method to measure water diffusion in the aligned lamellar phase of egg phosphatidylcholine (egg PC)-water.

Evidence that water forms a close association with phospholipid model membranes was originally derived from differential scanning calorimetry (Chapman et al., 1967). Thermograms for PC-water mixtures reveal that 10 waters per lipid do not freeze at 0°C. The implied binding of water to phospholipid was subsequently elaborated on by ^{2}H NMR (Salsbury et al., 1972; Finer, 1973; König et al., 1994; Volke et al., 1994; Hsieh and Wu, 1995). The majority view is that the first few water molecules bind tightly to the phosphate group and are motionally restricted; and that there is rapid exchange with other water, termed "trapped" or "quasi-free," in the aqueous interlamellar space between adjacent bilayers. Powder pattern spectra recorded for PC-^2H_2O systems in the lamellar phase confirm the anisotropic nature of bound water motion (Finer, 1973; Volke et al., 1994). The perpendicular to the membrane surface is shown to be an axis for reorientation by the $P_2(\cos\theta)$ dependence on orientation seen for the quadrupolar splitting from samples aligned between glass plates, where θ is the angle between the normal to the plates and the applied magnetic field (Pope and Cornell, 1979). Models of water binding have been proposed, although precise details are open to debate (Finer, 1973; Cornell et al., 1974; Gawrisch et al., 1978; Volke et al., 1994).

In contrast, there have been relatively few investigations of water diffusion in model membranes. The diffusion coefficients measured in PC-water systems are in the range of 1×10^{-10} m^2 s^{-1} to 20×10^{-10} m^2 s^{-1} depending on water concentration, temperature, and bilayer composition (Lange and Gary Bobo, 1974; Inglefield et al., 1976; Lindblom et al., 1977; Chan and Pershan, 1978; König et al., 1994; Volke et al., 1994). They are less than in bulk water. This reflects that bound and trapped water contribute to the measurement, which is a population-weighted summation from all water environments. Slow lateral diffusion of lipid on the order of 10^{-12} m^2 s^{-1} (Marsh, 1990) is a determinant of the rate at which bound water diffuses, whereas the translational movement of trapped water is likely to be impeded by lipid polar groups that protrude into the interlamellar aqueous region. Another factor that violates free diffusion is permeation through lipid bilayers. The nature of the dependence of water diffusion in PC-water samples on water concentration is controversial. Monotonic variation in which the diffusion coefficient increases with increasing

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water content has been observed in 1,2-dipalmitoylphosphatidylcholine (DPPC)-water and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC)-water (Lindblom et al., 1977; Chan and Pershan, 1978; Volke et al., 1994). However, unusual behavior in which a dramatic fall in water diffusion occurs after a small increase in the amount of water at a specific content was reported for egg PC-water and egg PC/cholesterol (1:1 molar)-water (Lange and Gary Bobo, 1974; Inglefield et al., 1976).

A study of the dependence on water concentration of water diffusion in the lamellar phase of egg PC-water aligned between glass slides is described here. The measurements were made noninvasively by the ^{1}H-NMR PFGSE approach (Kärger et al., 1988; Lindblom and Orädd, 1994). A major virtue of the investigation is the use of aligned samples. All lipid lamellae in an aligned sample possess the same orientation relative to the direction of the applied magnetic field gradient, which monitors diffusion in the PFGSE experiment. It is a crucial point, because water diffusion is anisotropic with respect to the lipid bilayer. Specifically, on the time scale of the measurement (~10 ms) lipid bilayers are a barrier to water diffusion perpendicular to the bilayer, which severely complicates interpretation of work on nonaligned systems.

MATERIALS AND METHODS

Sample preparation

Egg PC (grade 1) was purchased from Lipid Products (South Nutfield, UK). A chloroform/methanol solution typically containing 100 mg egg PC was dispensed into a 10-mm OD glass tube. The solvent was removed with a stream of nitrogen followed by vacuum pumping. An appropriate volume of triply distilled water was then added and the sample sealed under nitrogen. Thorough mixing was accomplished by repeatedly (~20 times) centrifuging the mixture back and forth through a narrow constriction (≤1 mm) in the tube.

The orientation technique consists of shearing a small amount of lipid-water mixture between glass plates, which produces a multilamellar layer of single alignment with the optical axis (bilayer normal) perpendicular to the plates (de Vries and Berendsen, 1969). In this manner, aligned egg PC-water samples at six different levels of hydration in the concentration range n = 4.9 mol water/lipid to n = 18.6 mol water/lipid were prepared. A sample was comprised of a stack of ~45 glass slides (10 mm × 6 mm × ≈0.1 mm), enclosing 44 aligned layers. The thickness of the layers was estimated to be ~10 μm, which corresponds to ~2000 bilayers. Each stack was assembled and sealed in a 10-mm NMR tube in a nitrogen atmosphere where the relative humidity was equivalent on the adsorption isotherm to the water content of the egg PC-water mixture from which the sample was derived (Elworthy, 1961). This minimized the loss of water during the alignment procedure. To prevent movement within the NMR tube, the stacks were constructed to just fit inside the cross section of the tube with the plates parallel to the axis.

NMR

A Bruker B-KR 332s pulsed NMR spectrometer and an AEI RS2 electromagnet operating at 1.4 T, corresponding to a ^{1}H-NMR resonant frequency of 60 MHz, were used. The 90° pulse length was on the order of 3.0 μs. Sample temperature was regulated to within ±0.5°C by a Bruker B-ST 100/700 temperature controller. The orientation of aligned samples with respect to the direction of the applied magnetic field B_0 was set to an accuracy of ±1° with the aid of a simple goniometer.

Self-diffusion coefficients D were measured by the PFGSE method (Stejskal and Tanner, 1965). This experiment introduces two identical field gradient pulses, one on either side of the 180° pulse, of magnitude G and width δ into a spin echo sequence 90°_x-τ-180°_y-acquire-delay. Diffusion that occurs in the time Δ between the field gradient pulses causes incomplete refocusing of the spin echo, which is attenuated according to

$$A(G) = A(0)\exp[-\gamma^2G^2\delta^2(\Delta - \delta/3)D] \qquad (1)$$

where γ is the gyromagnetic ratio. Here, G was varied to measure D. A homebuilt diffusion unit generated field gradient pulses of up to 2.5 T m^{-1} in an oppositely wound arrangement of Helmholtz coil pairs mounted on a standard Bruker probe. Fine adjustment of the width of the first field gradient pulse ensured exact matching. Maximal height for the spin echo, which was observed "single shot," provided the criterion. The measurements were calibrated relative to the diffusion coefficient D_w of pure water at 25°C, and absolute values stated assume $D_w = 2.3 \times 10^{-9}$ m^2 s^{-1} (Stejskal and Tanner, 1965); they possess an uncertainty of ±10%. Experimental parameters were usually τ = 12 ms, δ = 4 ms, and Δ = 12 ms.

Transverse relaxation was monitored with the Carr Purcell Meiboom Gill (CPMG) sequence 90°_x-(τ-180°_y-echo height acquire)$_n$-delay (Carr and Purcell, 1954; Meiboom and Gill, 1958). A Data Laboratories DL 102 computer-averaged transient recorder was employed to signal average, and 128 repetitions of the sequence were usually accumulated.

RESULTS

The results of a ^{1}H-NMR PFGSE experiment on an egg PC-water mixture containing n = 9.5 mol water/lipid at 25°C are presented in Fig. 1 as a semilogarithmic plot of echo attenuation $A(G)/A(0)$ vs. G^2, the square of the field gradient pulse amplitude. The graph is clearly nonlinear, which is symptomatic of the anisotropy of water diffusion with respect to lipid lamellae. At the water concentration of

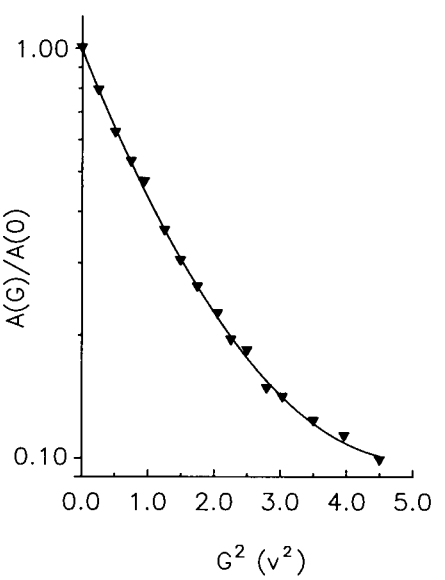

FIGURE 1 PFGSE diffusion plot of echo attenuation $A(G)/A(0)$ vs. G^2 square of field gradient pulse amplitude for egg PC-water mixture (n = 9.5 mol water/lipid) at 25°C. The field gradient was sampled as a voltage across a 0.2 Ω monitor resistance in series with the field gradient coils. Experimental parameters were τ = 12 ms, Δ = 12 ms and δ = 4 ms.

the sample, an egg PC-water mixture adopts a lamellar phase consisting of a random orientational distribution of lamellar domains (Small, 1967). The diffusion plot is consequently a superposition from domains at all angles relative to the direction of the field gradient G, which coincides with the magnetic field B_o. It appears curved when water diffusion is highly anisotropic, i.e., $D_{\parallel}/D_{\perp} \gg 1$ where the respective subscripts specify parallel and perpendicular to the plane of the bilayer. In principle $D_{\parallel}$ can be extracted by simulation, but not without assumptions such as the size of domains (Callaghan et al., 1983). These problems were avoided in the present work by employing aligned samples, which unambiguously define the direction of diffusion observed.

An appreciation of ^{1}H-NMR transverse relaxation in aligned egg PC-water is a prerequisite to measurement of water diffusion by PFGSE. Fig. 2 shows the decay of spin echo height recorded with the CPMG sequence for an aligned sample placed at different angles relative to the applied magnetic field B_o. It is important to recognize two features. The first is that the relaxation detected is a summation of a rapid decay from lipid and, because of appreciably greater mobility, a slower decay from water. Reflecting a range of restricted motions within the molecule, the lipid decay is multicomponent. The water decay is rendered single component by fast exchange between bound and trapped environments. A $|P_2(\cos\theta)|$ dependence on orientation with respect to B_o for the rate of relaxation of the entire decay is the second feature. Residual static, dipolar interactions are responsible. They dominate spin-spin relaxation for lipid and water because both molecules undergo anisotropic reorientation about the bilayer normal. Analogous behavior has been reported in earlier work on lyotropic liquid crystalline systems of single alignment (de Vries and Berendsen, 1969; Johansson and Drakenberg, 1971; Pope and Cornell, 1979; van der Leeuw and Stulen, 1981).

The time scale over which transverse magnetization persists is, in particular, longest when the optical axis is at the magic angle $\theta = 54°44'$ (Fig. 2 *B*). Because the intensity of the spin echo is maximized, this orientation is the optimum for performing PFGSE experiments. A diffusion plot recorded at $\theta = 55°$ for an aligned egg PC-water sample containing $n = 18.6$ mol water/lipid is presented in Fig. 3. Lipid and water contribute to the spin echo, so that the semilogarithmic graph is two component. Quickly diffusing water produces the initial steep rate of attenuation at low G^2,

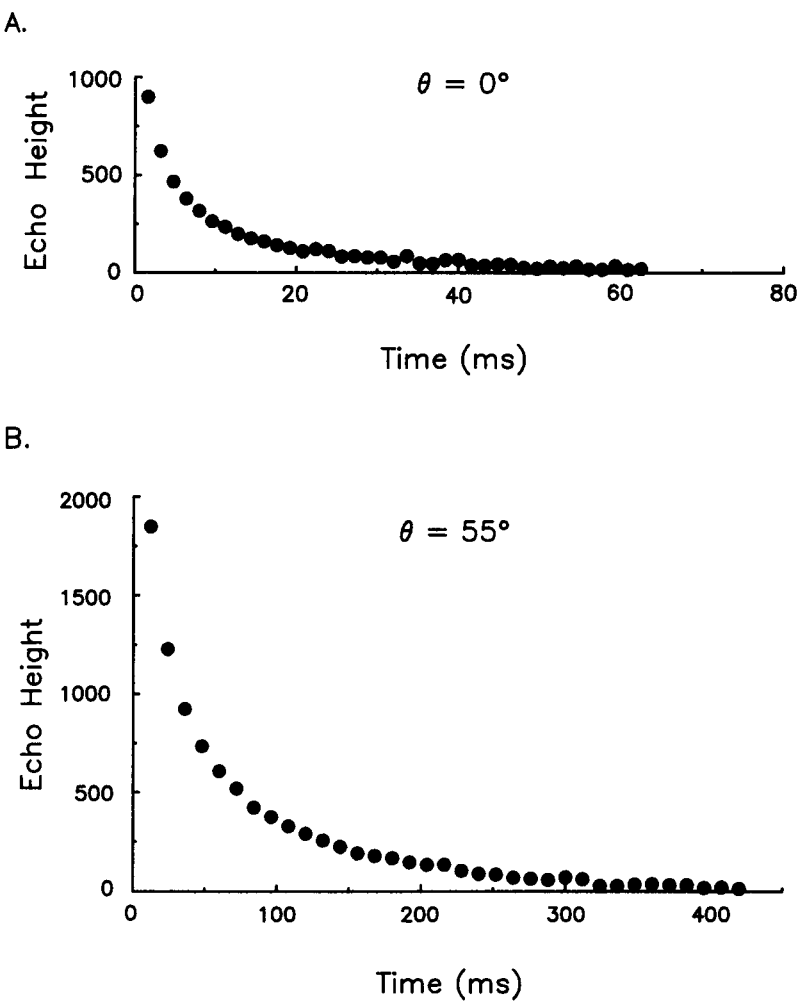

FIGURE 2 ^{1}H-NMR transverse relaxation in aligned egg PC-water ($n = 9.5$ mol water/lipid) at 25°C observed by CPMG for two orientations of optical axis with respect to magnetic field B_o. (*A*) $\theta = 0°$; (*B*) $\theta = 55°$.

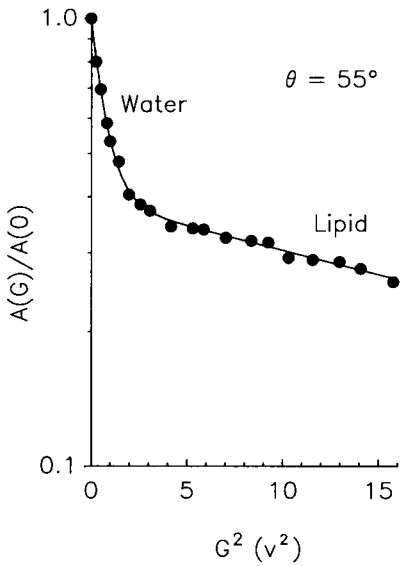

FIGURE 3 PFGSE diffusion plot of echo attenuation $A(G)/A(0)$ vs. G^2 square of field gradient pulse amplitude for aligned egg PC-water (n = 18.6 mol water/lipid) at 25°C with the optical axis at the magic angle θ = 54°44′ with respect to the magnetic field B_o. The line drawn through the data is a nonlinear, least-squares fit to two components, which gives diffusion coefficients $D = 2.8 \times 10^{-10}$ m^2 s^{-1} for water and $D_L = 8 \times 10^{-12}$ m^2 s^{-1} for lipid. Experimental conditions were identical to Fig. 1.

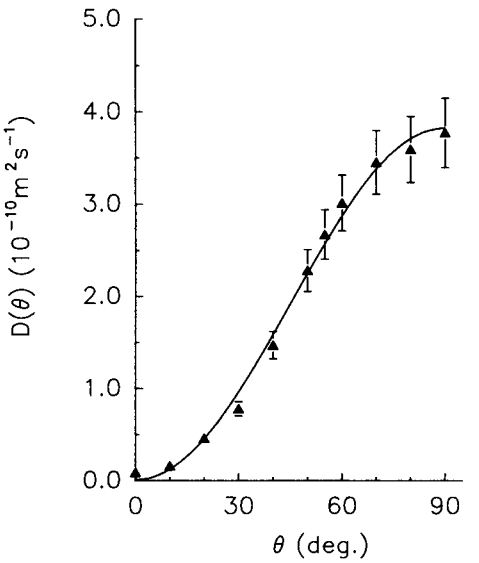

FIGURE 4 Water diffusion coefficient $D(\theta)$ vs. θ orientation of the optical axis relative to the field gradient G for aligned egg PC-water (n = 18.6 mol water/lipid) at 25°C. The fit of Eq. 2 to the data has $D_{\|} = 3.8 \times 10^{-10}$ m^2 s^{-1} and $D_{\perp} = 1.0 \times 10^{-12}$ m^2 s^{-1}. The error bars are ±10%, representing a lower limit to uncertainty that increases at orientations away from the magic angle θ = 54°44′.

whereas much more slowly diffusing lipid dominates the later stages at high G^2. The respective diffusion rates may be extracted by a nonlinear, least-squares fit to a biexponential, and the line drawn through the data was obtained with Mathematica (Wolfram Research, Inc., Champaign, IL). A lateral diffusion coefficient $D_L \approx 8 \times 10^{-12}$ m^2 s^{-1} was evaluated for lipid that, although appropriate for liquid crystalline PC (Marsh, 1990), cannot be considered better than an order of magnitude estimate as it is based on only a small amount of echo attenuation. The water diffusion coefficient that the fit yields is $D = 2.7 \times 10^{-10}$ m^2 s^{-1}. A reassuring aspect of the analysis is the closeness that exists between the ratio of the intensities of lipid:water identified from the diffusion plot with their relative intensity in the CPMG decay.

In the experimental arrangement employed, the field gradient G was fixed in the same direction as the static magnetic field B_o. This defines the direction in which diffusion is monitored and, unless the optical axis of an aligned sample is perpendicular to B_o, does not coincide with the bilayer plane and $D_{\|}$. The water diffusion coefficient observed at the magic angle in Fig. 3, thus, is a function of both $D_{\|}$ and $D_{\perp}$. Specifically,

$$D(\theta) = D_{\|}\sin^2\theta + D_{\perp}\cos^2\theta \qquad (2)$$

gives the water diffusion coefficient $D(\theta)$ when the optical axis makes angle θ with B_o (Lindblom and Orädd, 1994). The applicability of the equation is illustrated in Fig. 4 by the fit to measurements of $D(\theta)$ vs. θ for an aligned egg PC-water sample containing n = 18.6 mol water/lipid. There is good agreement, despite the deterioration in signal: noise ratio and substantially greater uncertainty associated with the diffusion experiment at orientations other than the magic angle. By confirming $D_{\|}/D_{\perp} \gg 1$, moreover, the data demonstrate that $D_{\perp}$ may be ignored in deriving $D_{\|}$ from the water diffusion coefficient measured at the magic angle; i.e., $D(\theta) = D_{\|}\sin^2\theta$ where $\sin^2\theta = 2/3$ for θ = 54°44′. Observations on other samples support the same conclusion throughout the water concentration studied. An identical angular correction factor was implicitly assumed in obtaining the lateral diffusion coefficient D_L of egg PC, which does not undergo diffusion perpendicular to the bilayer plane, for the lipid component in Fig. 3 (Lindblom and Orädd, 1994).

Water diffusion coefficients $D_{\|}$ in aligned egg PC-water were determined from PFGSE experiments performed at the magic angle for water concentrations between n = 4.9 mol water/lipid and n = 18.6 mol water/lipid. The time delay Δ between field gradient pulses, which specifies the interval over which diffusion is followed, was usually 12 ms. No change in $D_{\|}$ was revealed on increasing Δ to an upper limit of 27 ms, indicating that water diffusion parallel to lipid lamellae is unrestricted on this time scale. Fig. 5 shows the variation with water content recorded for $D_{\|}$ at 25°C. As can be seen, the rate of water diffusion smoothly rises from 1.2×10^{-10} m^2 s^{-1} to 4.0×10^{-10} m^2 s^{-1} with addition of water. The coefficients, which are on the order of a factor of 10 slower than pure water, fall within the range previously reported for PC-water systems in the liquid crystalline phase (Lange and Gary Bobo, 1974; Inglefield et al., 1976; Lindblom et al., 1977; Chan and Pershan, 1978; Volke et al., 1994).

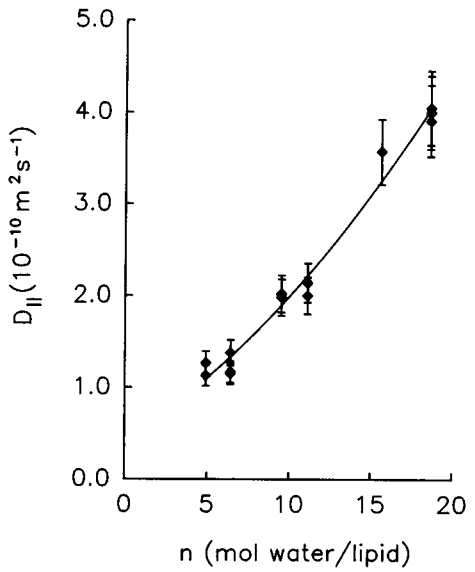

FIGURE 5 Water diffusion coefficient $D_{||}$ as a function of water concentration in aligned egg PC-water at 25°C.

The dependence on temperature between 1°C and 32°C of water diffusion $D_{||}$ in oriented egg PC-water is presented in Fig. 6 in the form of an Arrhenius plot. Several water concentrations and, for comparison, diffusion coefficients obtained in pure water (Simpson and Carr, 1958) are included. Activation energies E_A calculated decrease with increasing water content from E_A = 28.5 kJ mol^{-1} at n = 6.4 mol water/lipid to E_A = 23.0 kJ mol^{-1} at n = 18.6 mol water/lipid. The values are higher than E_A = 20.9 kJ mol^{-1} evaluated for pure water.

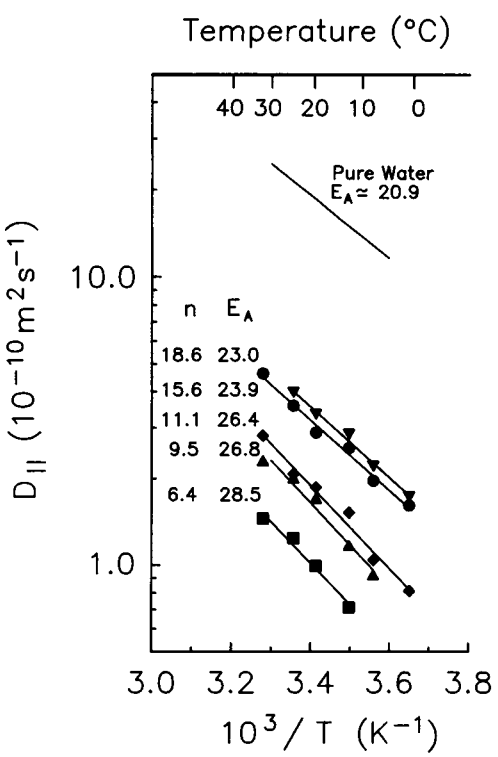

FIGURE 6 Temperature dependence of water diffusion $D_{||}$ in aligned egg PC-water. Water concentration n (mol water/lipid) and corresponding activation energy E_A (kJ mol^{-1}) are indicated. The data for pure water are taken from Simpson and Carr (1958).

DISCUSSION

Highly anisotropic diffusion of water with respect to the plane of the lipid bilayer ($D_{||}/D_{\perp} \gg 1$) is evident from the distinct nonlinearity of the diffusion plot shown in Fig. 1 for an egg PC-water mixture. The PFGSE experiment for such a sample observes the attenuation $A(G)/A(0)$ of a superposition of signals from water in a random orientational distribution of lamellar domains where, because of the anisotropy, the rate of diffusion monitored for each domain depends on its angle relative to the direction of the field gradient G. This complication, although apparently unrecognized in some early studies of nonoriented lipid-water samples (Blinc et al., 1970; Inglefield et al., 1976), has previously been discussed (Lindblom et al., 1977; Callaghan et al., 1983; Volke et al., 1994). Notably, curved diffusion plots in accordance with experimental data were simulated by Callaghan et al. (1983) to describe the diffusion of ^2H_2O in a potassium palmitate-^2H_2O mixture. In work on systems more closely related to the current study, surprisingly, linear diffusion plots were reported for egg PC/cholesterol (1:1)-water and POPC-water mixtures (Inglefield et al., 1976; Volke et al., 1994). The implication that water diffusion is characterized by low anisotropy with respect to these membranes seems unlikely. More probable explanations include the presence of defects in bilayer structure via which water can cross the membrane, and/or that there is a preponderance of lamellar domains of small lateral dimension ($\ll$5 μm) between which fast exchange of water occurs. Either eventuality could be sensitive to the method of sample preparation. This illustrates the problems associated with interpretation of PFGSE data for nonoriented samples, which are avoided when lamellar phase samples of single alignment are studied as advocated here.

Confirmation that the anisotropy of water diffusion relative to the egg PC bilayer is large is provided by Fig. 4. Measurements of diffusion coefficient $D(\theta)$ made as a function of orientation with an aligned egg PC-water sample unambiguously establish that water diffusion parallel to the bilayer (θ = 90°) is much faster than perpendicular to the bilayer (θ = 0°), i.e., the fit to Eq. 2 in Fig. 3 has $D_{||}/D_{\perp} \approx$ 380. The data, moreover, demonstrate that $D_{\perp}$ = 0 may be assumed when calculating $D_{||}$ from $D(\theta)$ essentially without introducing error. The slow rate implied for water diffusion through the bilayer is consistent with the water permeability coefficients P reported for egg PC (Finkelstein, 1987). They are on the order of $10^{-6} - 10^{-5}$ ms^{-1}, which corresponds to a diffusion coefficient $D \leq 10^{-13}$ m^2 s^{-1}. The possibility that the water diffusion monitored perpendicular to the bilayer in the PFGSE experiment is re-

stricted by the glass slides used to align the sample, rather than by lipid lamellae, is discounted. The thickness of the layers of lamellar phase between glass slides was estimated to be ≈ 10 μm, which is a factor of 4 greater than the distance water would be expected to diffuse in time $\Delta = 12$ ms between field gradient pulses if the condition $D_{\parallel} = D_{\perp}$ were to apply.

Fig. 4 represents the first comprehensive description of the angular variation of water diffusion in an aligned lipid-water system. Previous estimates of anisotropy $D_{\parallel}/D_{\perp}$ were usually based on observations made at only two orientations, typically $\theta = 0°$ and $\theta = 90°$. In agreement with the high anisotropy seen for egg PC, $D_{\parallel}/D_{\perp} \approx 50$ was determined for liquid crystalline DPPC bilayers (Ukleja and Doane, 1980), while negligible diffusion perpendicular to the bilayer was detected for POPC (Volke et al., 1994). Large values $D_{\parallel}/D_{\perp} \geq 25$ and $D_{\parallel}/D_{\perp} \approx 34$ were similarly measured for the diffusion of ^1H_2O and ^2H_2O, respectively, relative to potassium palmitate bilayers (Ukleja and Doane, 1980; Callaghan et al., 1983); and little or no water diffusion across the bilayer of potassium oleate was deduced from a comparison of diffusion coefficients obtained with aligned and nonaligned samples (Chien et al., 1974). In contrast, low anisotropy was observed in magnetically aligned samples prepared from perfluoro-alkyl surfactants with short chains. Water diffusion coefficients $D_{\parallel}$ and $D_{\perp}$ that are of comparable magnitude were measured with respect to perfluorooctonoate and perfluorononanoate bilayers (Tiddy et al., 1974; Chidichimo et al., 1988; Holmes et al., 1993; Furó and Jóhannesson, 1996). The presence of bilayer defects through which water diffuses was suggested to be the reason, whereas an earlier proposal has the relatively small thickness of the bilayer enabling water to cross easily. The identification of the lamellar phase in cesium pentadecafluorooctonoate-water as consisting of planar arrays of discoid micelles, instead of a continuous bilayer, favors the more recent rationale (Boden et al., 1987).

The water diffusion coefficients $D_{\parallel}$ plotted in Fig. 4 for aligned egg PC-water at 25°C clearly vary smoothly with water content. As can be seen the diffusion, which is appreciably slower than in bulk water where $D_W = 2.3 \times 10^{-9}$ m^2 s^{-1} (Stejskal and Tanner, 1965), increases from $D_{\parallel} = 1.2 \times 10^{-10}$ m^2 s^{-1} to $D_{\parallel} = 4.0 \times 10^{-10}$ m^2 s^{-1} over the water concentration range $n = 4.9$ mol water/lipid to $n = 18.6$ mol water/lipid. Such behavior may be qualitatively explained in terms of the properties of water in the lamellar phase. The binding of water to lipid and the obstruction lipid polar groups cause to diffusion within the aqueous layer reduce the rate of diffusion compared with bulk, free water. By introducing less tightly bound water and by enlarging the thickness of the aqueous layer, faster diffusion is expected to accompany the addition of water.

A monotonic dependence on water content is the consensus of prior, sometimes disparate work on water diffusion in lipid-water systems. Water diffusion coefficients that rise from 2×10^{-10} m^2 s^{-1} at $n = 5$ mol water/lipid to 8×10^{-10} m^2 s^{-1} at $n = 30$ mol water/lipid were measured in PFGSE experiments on POPC-water mixtures at 28°C (Volke et al., 1994). When the same method was applied to DPPC-water mixtures in the liquid crystalline state, water diffusion coefficients varying from 3.8×10^{-10} m^2 s^{-1} to 7.0×10^{-10} m^2 s^{-1} were obtained over the water concentration range $n = 7$ mol water/lipid to $n = 15$ mol water/lipid (Lindblom et al., 1977); while the variation described for aligned DPPC-water at 70°C on the basis of optical measurements consists of an order of magnitude increase in water diffusion coefficient parallel to lamellae from $D_{\parallel} = 1 \times 10^{-10}$ m^2 s^{-1} to $D_{\parallel} = 2 \times 10^{-9}$ m^2 s^{-1} between 10 wt % ($n = 4.5$) and 15 wt % ($n = 7.2$) water, followed by little change thereafter (Chan and Pershan, 1978). Faster water diffusion is the trend seen, furthermore, by PFGSE and neutron-scattering techniques in aligned and nonaligned perfluorooctonoate-water samples as water is introduced (Tiddy et al., 1974; Holmes et al., 1993).

Radioactive tracer studies of ^{3}HHO diffusion in egg PC-water mixtures provide an exception to the tendency for addition of water to increase the rate of water diffusion (Lange and Gary Bobo, 1974; Gary-Bobo and Rigaud, 1976). In particular, an abrupt reduction from 4.5×10^{-10} m^2 s^{-1} to 1.5×10^{-10} m^2 s^{-1} in ^{3}HHO diffusion coefficient at 25°C was reported to accompany an increase in water content beyond 21 wt % water ($n = 11.2$). A sudden fourfold decrease in water diffusion from 4.5×10^{-10} m^2 s^{-1} to 1.1×10^{-10} m^2 s^{-1} between 25.5 wt % ($n = 21.8$) and 30.5 wt % ($n = 28.0$) water was similarly recorded in egg PC/cholesterol (1:1)-water mixtures at 20.5°C with the PFGSE technique (Inglefield et al., 1976). The proposal that an alteration in headgroup conformation at specific hydration causes greater stearic hindrance to water diffusion in the aqueous layer (Lange and Gary Bobo, 1974) is unequivocally refuted by the continuously rising water diffusion coefficients presented for aligned egg PC-water in Fig. 4. Investigations of headgroup conformation and water binding, moreover, have failed to reveal a discontinuity commensurate with the proposed change from a bent configuration, in which the phosphorylcholine group is parallel to the bilayer, to an extended configuration, in which the phosphorylcholine group is perpendicular to the bilayer (Bechinger and Seelig, 1991; Ulrich and Watts, 1994; Volke et al., 1994). A mesomorphic rearrangement whereby lipid lamellae fold to form closed structures that constrain the diffusion of water is a probable explanation. Alignment between glass plates precludes such structural complications, whereas, because a precipitous drop in water diffusion is not always seen in studies of nonoriented samples, the precise manner of sample mixing and time scale over which diffusion is monitored are presumably critical factors with lipid-water mixtures. These considerations further highlight the experimental advantage of well-defined, aligned samples.

Various models for the organization and dynamics of water associated with phospholipid membranes have been put forward (Finer, 1973; Cornell et al., 1974; Gawrisch et al., 1978; Volke et al., 1994). A model presented by Finer

(Finer, 1973; Finer and Darke, 1974) to interpret quadrupolar splittings obtained for egg PC-^2H_2O mixtures in terms of the filling of a series of hydration "shells" may be directly adapted to the water diffusion in aligned egg PC-water monitored here. The treatment assumes each hydration shell is completely filled before population of the next, less strongly bound, environment commences; the motion of all water molecules in a given shell is equivalent; and there is fast exchange between shells. The resultant splitting $\Delta\nu$ is a population-weighted average, which when the jth shell is partly full can be written

$$\Delta\nu = \frac{1}{n}\sum_{i=1}^{j-1} N_i(\Delta\nu_i - \Delta\nu_j) + \Delta\nu_j \qquad (3)$$

where N_i and $\Delta\nu_i$ designate, respectively, the capacity and characteristic splitting of the ith shell. This equation predicts that a plot of $\Delta\nu$ vs. $1/n$ follows a series of linear regions, each corresponding to the stripping of a hydration shell. For egg PC-^2H_2O mixtures, separate linear regions attributed to bound water undergoing anisotropic reorientation ($n \leq 12$), isotropically reorienting water trapped between lipid lamellae ($12 < n \leq 23$) and bulk water ($n > 23$) were distinguished. Confirmation was provided in subsequent work (Gawrisch et al., 1985). The assignment of water into distinct hydration shells in which molecular motion remains unchanged on varying water content has been criticized (Volke et al., 1994). An alternative hypothesis that was recently advocated has the bilayer surface exerting an exponentially decaying influence on water orientation and dynamics with increasing hydration. Nevertheless, the merit of the Finer model is asserted by successful agreement with experiment.

To apply the same approach to water diffusion coefficients $D_\parallel$ measured in aligned egg PC-water, each hydration shell is further assumed to have its own diffusion coefficient D_i. The expression describing the diffusion coefficient D when the jth shell is only partially occupied, in direct analogy with Eq. 3, is then

$$D = \frac{1}{n}\sum_{i=1}^{j-1} N_i(D_i - D_j) + D_j\,. \qquad (4)$$

Thus, a series of linear regions is also predicted for a plot of D vs. $1/n$. Fig. 7 shows that linear regions corresponding to the bound and trapped water shells identified by Finer may be ascribed to the diffusion data. Extrapolation to the ordinate axis yields an intrinsic diffusion coefficient of 7.5×10^{-10} m^2 s^{-1} for trapped water that, although characterized as undergoing isotropic reorientation, is restricted compared to bulk, free water. The nonzero slope for bound water implies the presence of an innermost hydration shell with capacity $n \leq 5$, which is consistent with the nonzero slope seen for the bound water portion of the $\Delta\nu$ vs. $1/n$ graph for egg PC-^2H_2O mixtures.

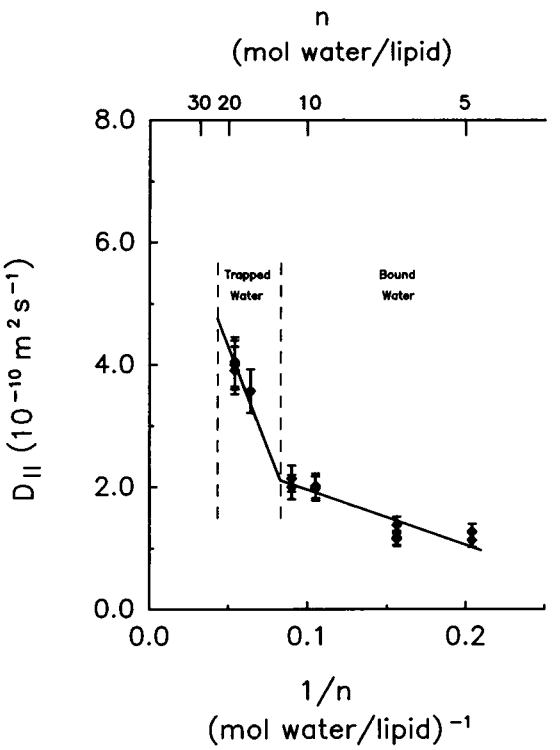

FIGURE 7 Variation of water diffusion $D_\parallel$ with $1/n$ reciprocal of water concentration for aligned egg PC-water at 25°C. The regions labeled bound and trapped water are the hydration shells identified by Finer (1973).

Arrhenius plots presented in Fig. 6 show that water diffusion $D_\parallel$ in aligned egg PC-water increases with temperature throughout the range of water concentration studied. As also indicated, activation energies that decrease from E_A = 28.5 kJ mol^{-1} at n = 6.4 water/lipid to E_A = 23.0 kJ mol^{-1} at n = 18.6 mol water/lipid were calculated. All of the values exceed $E_A \approx 20.9$ kJ mol^{-1} evaluated for pure water, which was anticipated because of the binding of water to egg PC. Because less tightly bound exists at higher water content, in addition, the activation energy is expected to decrease with introduction of water. This offers a qualitative rationale for the concentration dependence of activation energies obtained here.

Activation energies previously quoted for water diffusion in PC-water systems, although relatively rare, are generally greater than bulk water. An apparent activation energy of 8.0 kcal mol^{-1} (33.5 kJ mol^{-1}) was estimated for ^{3}HHO diffusion in egg PC-water mixtures between 22 wt % (n = 11.9) and 26 wt % (n = 14.8) water (Rigaud et al., 1972). The same trend as in the current work was seen in aligned DPPC-water, where activation energies that decrease from ~0.4 eV (38.5 kJ mol^{-1}) at 10 wt % water (n = 4.5) to 0.2 eV (19.3 kJ mol^{-1}) at 25 wt % water (n = 13.6) were evaluated (Chan and Pershan, 1978). However, the factor of 2 change that was claimed is somewhat larger than the ~20% reduction in aligned egg PC-water. A dependence on hydration, analogously, has been recorded for the activation energy of lipid lateral diffusion in PC bilayers (Kuo and

Wade, 1979). Activation energies that go from 18.6 kcal mol^{-1} (77.9 kJ mol^{-1}) at 15 wt % ^2H_2O (n = 6.5) to 13.2 kcal mol^{-1} (55.3 kJ mol^{-1}) at 40 wt % ^2H_2O (n = 24.5) were measured for lipid diffusion by PFGSE experiments on aligned DPPC-^2H_2O.

In conclusion, the results on water diffusion in egg PC-water presented here demonstrate that water diffusion is highly anisotropic $D_\parallel D_\perp \gg 1$ with respect to the lipid bilayer and that water diffusion parallel $D_\parallel$ to the bilayer increases continuously with water concentration. The latter finding, made with samples of single alignment, contradicts earlier studies of egg PC-water mixtures that saw an abrupt reduction in water diffusion at a certain water content. A structural rearrangement of domains within the lamellar phase, which does not occur when aligned between glass plates, is the probable origin of the discrepancy. The advantage of studying water diffusion in oriented versus nonoriented lipid-water systems is definitively illustrated.

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