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WATER ORIENTATION AND MOTION IN PHOSPHOLIPID BILAYERS: A COMPARISON BETWEEN ^{17}O- AND ^{2}H-NMR

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^{17}O- and ^{2}H-NMR spectra were obtained of a lamellar phase of dipalmitoyl-3sn-phosphatidylcholine (DPL) and $D_2{}^{17}O$ with water content of 3–15 moles water/mole DPL, in the temperature range 20 to 80°C. In every case, the quadrupole splittings observed for ^{17}O were 6.6 times larger than those for ^{2}H. Therefore the two methods contain in principle the same information, but with less details from ^{17}O. On the other hand, dynamic information is easily obtained from ^{17}O linewidth data and complements the deuterium results.

1. Introduction

Phospholipid-water bilayers have received considerable attention as models for biological membranes. Since the role of water is important, many studies concerning its orientation and motion have been performed. Orientational studies were made almost exclusively by deuterium magnetic resonance [1,2]. In a recent communication [3] we have combined ^{2}H- and ^{17}O-NMR informations to derive the two order parameters describing the water orientation. The results turned out to be imprecise. In this paper therefore, we compare the results of ^{2}H- and ^{17}O-NMR rather than combine them, for a bilayer phase of dipalmitoyllecithin and water.

2. Experimental

Dipalmitoyllecithin (DPL) bilayers were prepared by thoroughly mixing DPL and water. DPL (1,2-dipalmitoyl-3sn-phosphatidylcholine) was obtained from Fluka AG, Buchs, and was vacuum dried overnight before use. Water with 20% ^{17}O and 99.9% ^{2}H was purchased from Biogenzia Lemania SA, Lausanne, and was used without further purification. Approximately 500 mg of the mixture were weighted into a 8 mm o.d. ampoule constricted in the middle. The ampoule was sealed, and homogenization was achieved by centrifugation back and forth through the constriction at about 50°C. Measurements were carried out on the ampoules placed inside standard 10 mm NMR tubes.

^{17}O-NMR spectra were recorded at 27.1 MHz on a Brucker WP-200 (Bruker, Karlsruhe and Spectrospin, Zürich). ^{2}H-NMR spectra were recorded at 13.8 MHz on a Bruker HX-90. The temperature unit of the spectrometers was calibrated with a Hewlett-Packard thermometer 2802 A.

3. Theory

In a lamellar phase, the tumbling of the water molecules bound to the bilayer is anisotropic. Therefore the ^{17}O-NMR spectrum of $D_2{}^{17}O$ exhibits five equally spaced lines (^{17}O has spin 5/2) with an intensity ratio 5 : 8 : 9 : 8 : 5 [4]. The ^{2}H-NMR spectrum shows the well-known doublet. These characteristic splittings arise from the interaction of the quadrupolar moment of the nucleus (^{17}O or ^{2}H) with the electric field gradient in the water molecule. The observed quadrupole splitting Δ between two adjacent lines is given by [5]:

$$\Delta = \frac{1}{2I(2I-1)} \chi \, [3S_{zz} + \eta(S_{xx} - S_{yy})], \qquad (1)$$

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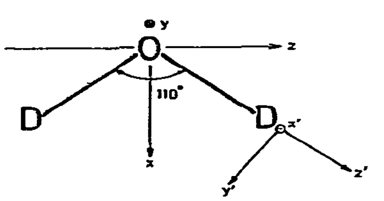

Fig. 1. The unprimed axis system chosen for the ^{17}O nucleus and the primed system for the deuterium nucleus in D_2O represent the principal axis system of the electric field gradient tensor [7,8].

where I is the spin, χ and η are the quadrupole coupling constant and the asymmetry parameter, respectively, of the nucleus; S_{xx}, S_{zz} and $S_{yy} = -(S_{xx} + S_{zz})$ are the order parameters of the water molecule with respect to the director [6] of the mesophase. Eq. (1) is valid only in the principal axis system of the electric field gradient tensor $\mathbf{V}$ at the nucleus and under the condition $|V_{zz}| \geqslant |V_{yy}| \geqslant |V_{xx}|$ [5]. The asymmetry parameter is defined as $\eta = (V_{xx} - V_{yy})/V_{zz}$ and the quadrupole coupling constant as $\eta = eQV_{zz}/h$, where eQ is the quadrupolar moment and h is Planck's constant. In fig. 1, the principal axis system for ^{17}O in water is unprimed, and that for ^2H is primed, where we assume that the electric field gradient in the liquid crystalline phase is nearly the same as for D_2O ice [7,8].

The order parameters of the two axis systems are related to each other by a tensorial transformation containing the direction cosines. For an axis a in the primed system, eq. (2) holds [5]:

$$S_a = \sum_{i,j} \cos \alpha_i^a \cos \alpha_j^a S_{ij}, \qquad (2)$$

where $i, j = x, y, z$ and α_i^a and α_j^a are the angles between axes a and i, and between a and j. Consequently, this transformation depends on the D—O—D angle in water, which is taken as 110° in fig. 1 [9].

4. Results and discussion

4.1. Orientational information

Typical ^{17}O-NMR spectra of the lamellar DPL $D_2{}^{17}O$ phase with 5 $D_2{}^{17}O$ molecules per DPL molecule are shown in fig. 2 at three different temperatures.

This mesophase does not align macroscopically in the magnetic field and gives rise to a powder-type spectrum, as is evident from the shape of the two outer

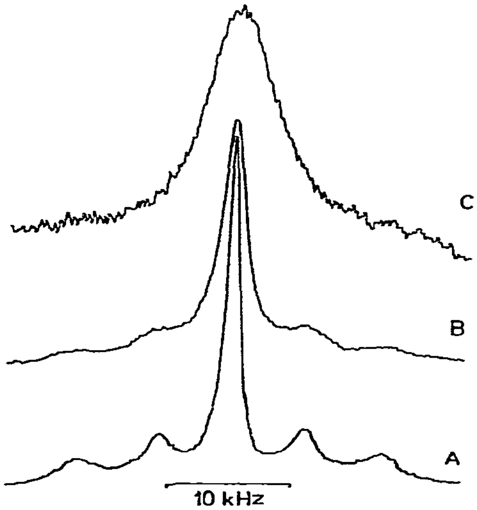

Fig. 2. ^{17}O-NMR spectra (27.1 MHz) of bilayers of dipalmitoyllecithin-$D_2{}^{17}O$ (20% ^{17}O) in a molar ratio 1 : 5 at 60°C (A), 50°C (B) and 20°C (C). 2000 scans were accumulated in 200 s; spectral width 50 kHz; pulse width 13 μs (90° pulse); line broadening 50 Hz.

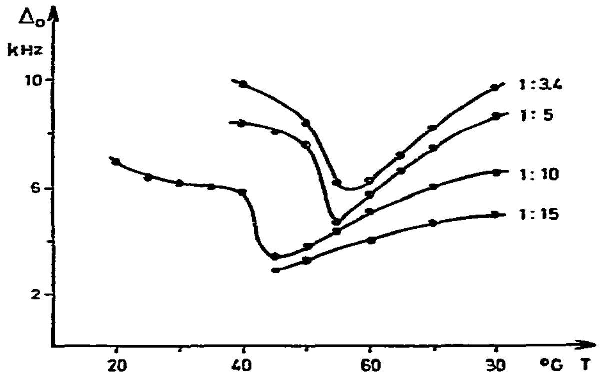

Fig. 3. Variation of the oxygen quadrupole splitting Δ_0 of DPL bilayers with temperature at four different molar ratios DPL : $D_2{}^{17}O$ = 1 : n (n = 3.4, 5, 10 and 15).

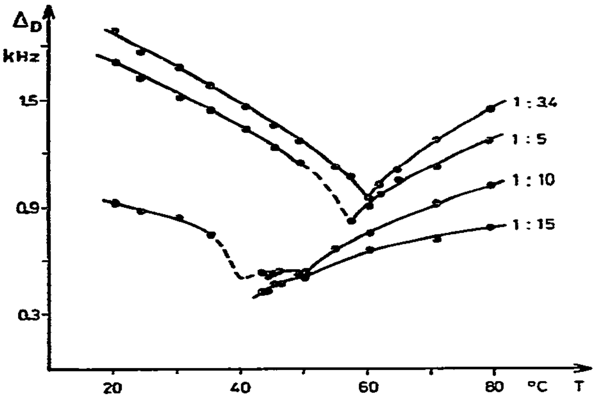

Fig. 4. Variation of the deuterium quadrupole splitting Δ_D of DPL bilayers with temperature at four different molar ratios DPL : $D_2{}^{17}O$ = 1 : n (n = 3.4, 5, 10 and 15). The dashed lines indicate regions of several coexisting phases.

pairs of lines. The ^{17}O quadrupole splittings Δ_O, extracted from the experimental spectra at various temperatures, are collected in fig. 3 for several DPL/water molar ratios.

The sample with 10 water molecules per DPL molecule is unique in giving a detectable ^{17}O quadrupole splitting over the entire temperature range of 20°C to 80°C. For all other samples, the splitting is no longer resolved below 40°C, because linewidth and splitting became comparable in size (see later, fig. 5).

In addition to the ^{17}O spectra, we have measured the ^2H spectra of the samples. The results for the ^2H quadrupole splittings Δ_D are shown in fig. 4.

An almost perfect agreement between the curves for Δ_O and Δ_D is obvious from a visual comparison of figs. 3 and 4. Indeed, only slight variation in the ratio Δ_O/Δ_D, found to be 6.6 ± 0.2, was noticed for the temperature range from 60 to 80°C. An explanation for this behaviour follows immediately from eq. (1) which describes the ^{17}O and ^2H quadrupole splitting. With the approximation of a perfect tetrahedral structure for the water molecule and of the two asymmetry parameters equal to 1 and 0 for ^{17}O and ^2H, respectively, eq. (1) reduces to:

$$\Delta_O = 1/10\, \chi_O\, (2S_{zz} + S_{xx}) \tag{3}$$

$$\Delta_D = 3/2\, \chi_D S_{z'z'}. \tag{4}$$

Eq. (4) reduces further to:

$$\Delta_D = 1/2\, \chi_D\, (2S_{zz} + S_{xx}) \tag{5}$$

by applying eq. (2) to $S_{z'z'}$ with tetrahedral angles. Combining eq. (3) with eq. (5) then yields:

$$\Delta_O/\Delta_D = 1/5\, \chi_O/\chi_D. \tag{6}$$

Eq. (6) shows that the ratio of the quadrupole splittings depends only on the quadrupole coupling constants and no longer on the two order parameters of the water molecule.

It should be mentioned that the approximations used are not too crude. Indeed, in various forms of ice, asymmetry parameters η are between 0.86 and 0.97 for ^{17}O, and around 0.1 for ^2H [10,11]. In bariumchlorate monohydrate, the corresponding parameters are 0.94 and 0.07 [9,12] and an H—O—H angle of 110° was determined [9]. It is therefore justified to derive the ratio of the quadrupole coupling constants χ_O/χ_D using eq. (6). For water in the liquid crystalline phase studied, a value of 33 ± 1 results for χ_O/χ_D. According to the literature, this ratio is almost independent of the amount of hydrogen bonding: For various forms of ice, χ_O/χ_D lies between 30.2 and 31.8 [10,11], in bariumchlorate monohydrate the value is 31.3 [9,12], whereas the ratio in the vapor phase evaluated as 33 is only slightly higher [13]. The value derived in this work is considered to agree satisfactorily with those just cited, taking into account the approximations used in our determination.

A consequence of this nearly constant Δ_O/Δ_D ratio is that ^{17}O spectra give the same information as ^2H spectra about the water orientation. This fact has been mentioned previously [3]. A large error resulted if the two independent order parameters S_{xx} and S_{zz} were determined simultaneously. Only the O—D axis order parameter $S_{OD} = 1/3\,(S_{xx} + 2S_{zz})$ is obtained with high accuracy, either from the ^2H splitting ($S_{OD} = 2/3\, \Delta_D/\chi_D$) or from the ^{17}O splitting ($S_{OD} = 10/3\, \Delta_O/\chi_O$). However, the deuterium method has the advantage that the quadrupole splitting is easier to detect, since the ratio splitting/linewidth is higher than for oxygen-17.

At this point, it should be mentioned that our deuterium results are in good agreement with those recently published by Ulmius et al. [1]. These authors constructed a phase diagram for the DPL—D_2O system

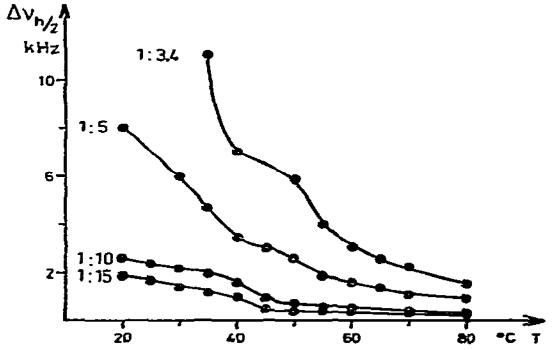

Fig. 5. Variation of the linewidth at half height $\Delta\nu_{h/2}$ of the central line, in the ^{17}O-NMR spectrum of DPL bilayers, with temperature at four different molar ratios DPL : $D_2{}^{17}O$ = 1 : *n* (*n* = 3.4, 5, 10 and 15).

by analysis of the quadrupole splitting and the shape of the deuterium spectra. For 8 to 12 water molecules per DPL, two or more coexisting phases gave rise to superimposed deuterium doublets. This is indicated by dashed lines in fig. 4. These details were not detectable in the corresponding ^{17}O spectra, presumably due to larger linewidth and poorer signal-to-noise ratio.

4.2. Dynamic information

The ^{17}O-NMR spectrum contains not only information about the water orientation but also about the water dynamics. The linewidth of the central peak $\Delta\nu_{h/2}$ is the easiest accessible dynamic parameter. The experimental results are given in fig. 5 for the temperature range 20 to 80°C and several molar ratios of DPL/$D_2{}^{17}O$. All curves have different slopes in the gel and liquid crystalline state, indicating a less hindered motion in the liquid crystalline state. The phase transition is clearly visible in the linewidth plot. The transition temperatures found are in good agreement with those determined from the quadrupole splittings, either in the oxygen-17 or the deuterium spectra (fig. 3 and 4).

From the linewidth of the central peak an average water correlation time τ_c can be calculated. Since the ^{17}O relaxation is dominantly quadrupolar, and the water orientation is small, the formula for the isotropic case [4]

$$\Delta\nu_{h/2} = \frac{1}{\pi T_2} = \frac{12}{125}\pi(1+\eta_O^2/3)\chi_O^2\tau_c \qquad (7)$$

may be used, where χ_O and η_O are the quadrupole coupling constant and the asymmetry parameter of the ^{17}O nucleus already introduced in eq. (1). If these parameters are assumed to have the same values as found in ice [7] ($\eta_O = 0.935$ and $\chi_O = 6.66$ MHz), the values obtained for τ_c from eq. (7) are in the range between 9 ps (for the 1 : 15 molar ratio phase at 80°C) and 640 ps (for the 1 : 3.4 molar ratio phase at 35°C). For comparison, isotropic pure water has a τ_c equal to 3 ps at room temperature and equal to 1 ps at 80°C.

The number of water molecules bound to a DPL molecule may be estimated from a plot of the linewidth $\Delta\nu_{h/2}$ versus the water concentration. The slope of the curves (not shown) changes drastically at about 6 water molecules per lipid molecule for all temperatures between 35°C and 80°C. Our result indicates that 6 water molecules can bind to a lecithin molecule. Hence, a correlation time for bound water of 20 ps at 80°C and at the highest water concentration studied (1 : 15 DPL/water molar ratio) is obtained, assuming rapid exchange between free (isotropically tumbling) and bound water. Lowering the water concentration results in a longer correlation time for the bound water molecules of 70 ps at 80°C and for 3.4 water molecules per lipid molecule. This change might be due to the increased overlap of the lipid head groups located on opposite sides of the aqueous layer when the layer becomes smaller.

The results derived in this study agree well with those of other investigations. Gottlieb et al. also found 6 lecithin-bound water molecules from a linewidth study of the water signal by proton NMR [14]. From a spin-lattice relaxation time measurement of the water deuterons, Cornell et al. [15] found two correlation times describing the water tumbling: The shorter of them, with a value of $\tau_c \leqslant 10^{-10}$ s for an axis parallel to the bisector of the D–O–D angle, is in agreement with our result. Using the same technique in a specifically deuterated DPL bilayer, Seelig et al. [16] determined a correlation time of 19 ps for the choline methyl group at 70°C and at high water concentration (40 water molecules per lipid molecule). This value compares well with the correlation time of 20 ps for bound water at 80°C and 15 water molecules per lipid molecule.

As mentioned previously [3], the linewidth of the two outer pairs of lines in the ^{17}O powder spectrum contains additional information about the water motion. However, the baselines of our experimental spectra were not sufficiently flat in most cases, preventing a simulation of the powder-type spectra with two additional linewidth parameters.

4.3. Temperature dependence of the observed parameters

In the variation of the observed quadrupole splittings and linewidths with temperature (see fig. 3, 4 and 5), one fact is unusual and merits therefore a special discussion: The observed quadrupolar splitting *increases* with increasing temperature in the liquid crystalline state. Because thermal motion would show an opposite trend, another effect must predominate. Two types of explanations have been proposed. The first one [1,17] supposes that melting the lipid chains at the phase transition creates new binding sites for water. The resulting redistribution of bound water would raise the average order. More recently, Abdolall et al. [18] have proposed another model based on purely geometrical parameters, which explains the same behaviour found in the simpler lamellar phase of potassium palmitate and water. At the phase transition, water is mainly hydrogen bonded to two neighboring carboxyl polar head groups. In this configuration, the order parameter of the O–D axis, $S_{OD} = 1/2 \langle 3\cos^2\theta - 1\rangle$ is close to zero (–0.006), because the angle θ between the O–D axis and the director of the lamellar phase is almost magic. Increasing the temperature results in breaking one of the two hydrogen bonds. The order parameter S_{OD} takes now a value of 0.169, assuming a D–O–D angle of 110°. The apparent order parameter of bound water becomes therefore much larger at higher temperature, although the system is less ordered.

The ^{17}O linewidth data (fig. 5) show that a dynamic perturbation occurs only at the phase transition. At higher temperatures the expected behaviour is observed. This indicates that the first explanation, assuming new binding sites, concerns only the phase transition. It does not explain the temperature dependence of the quadrupole splitting at high temperatures. Furthermore, it has been found by deuterium and phosphorus-31 NMR and neutron diffraction that the polar head groups are always parallel to the membrane surface and do not change significantly at the phase transition [19]. Therefore the hydration state of DPL should not change to a great extent during the phase transition. On the other hand, the geometrical model seems to apply generally to lamellar phases with simple polar head groups: In an investigation of the ternary system of Na-decanoate (30 wt %), n-decanol (36 wt %) and $D_2{}^{17}O$ (34 wt %), we found that the quadrupolar splitting shows the same trend and increases from 2 kHz at 10°C continuously to 11 kHz at 70°C. Even in complex polar head groups such as phosphatidylcholine in DPL, the observed temperature dependence of the splitting is very similar. This seems to indicate that several features of water binding in the simple systems apply also to the DPL case.

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