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(41) Hanlon and Major[22] have shown that, even at pH values corresponding to protonation of the adenine ring, ethylene glycol effectively prevents the stacking of 5'-AMP when the nucleotide concentration is in the ranges used in this study. At neutral or alkaline pH values this glycol effectively prevents stacking within polyriboadenylic acid (and AMP). Stacking involving the nucleoside polyphosphates ought to be even less likely. Corresponding data with tetramethylurea as the solvent are not available because of the absorbance of this and similar molecules at key regions in the ultraviolet spectrum.
(42) A possible mechanism for these shift changes may be the alteration of the torsional conformation resulting from the protonation of the base. Gorenstein and Kar[30] have demonstrated that such shifts in phosphoric diesters can arise from conformational changes.

Phosphorus-31 Chemical Shift Anisotropy in Unsonicated Phospholipid Bilayers

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Abstract: Phosphorus-31 NMR spectra of unsonicated bilayer dispersions of dipalmitoyl-3-*sn*-phosphatidylcholine are recorded at temperatures above and below the gel-to-liquid crystalline phase transition. Proton decoupling yields spectra characteristic of axially symmetric chemical shift anisotropy. A quantitative interpretation of proton decoupled and nondecoupled spectra yields accurate numbers for the chemical shift anisotropy and the proton–phosphorus dipole–dipole interactions.

Normal phosphorus-31 NMR spectra of unsonicated phospholipid bilayers at resonance frequencies up to 90 MHz show one broad line of approximately 50 ppm line width.[1,2] The line shape of the powder-type spectra is caused by (a) the chemical shift anisotropy of the phosphorus nuclei and (b) the proton–phosphorus dipole–dipole interactions. One possibility to distinguish between the two contributions is to perform the experiments at higher resonance frequencies, because only the chemical shift is dependent on the magnetic field strength.[3] An alternative method is to suppress the proton–phosphorus dipole–dipole interactions by means of a proton decoupling field. A first successful application of this method to phospholipid bilayers has been published.[4] Recently the static chemical shift tensors of some phospholipids and phospholipid constituents have also become available.[5] Since phosphorus-31 NMR may thus prove to be of increasing importance for the elucidation of the polar region of membranous structures, the purpose of this report is to give a quantitative interpretation of phosphorus-31 NMR spectra of unsonicated lipid bilayers under proton decoupled and nondecoupled conditions.

Experiment and Results

The experimental conditions are the same as described earlier.[4] Dipalmitoyl-3-*sn*-phosphatidylcholine (50 wt%) was thoroughly mixed with water (50 wt %) in a sealed ampule. About 500 mg of material was placed in a 10-mm NMR sample tube. ^{31}P-NMR measurements were made above and below the gel-to-liquid crystal phase transition at 41 °C. All spectra were recorded on a Bruker-Spectrospin HX-90 pulse-fourier-transform spectrometer at a resonance frequency of 36.4 MHz. Proton decoupling experiments were performed with a Bruker-Spectrospin B-FS-100 frequency synthesizer and modulation unit.

Figure 1 shows phosphorus-31 NMR spectra of coarse dispersions of phospholipid bilayers above and below the phase transition. The proton decoupled spectra (Figures 1A and 1C) have the line shapes anticipated for powder-type spectra with axially symmetric chemical shift anisotropy,[6] while the nondecoupled spectra are dominated by dipole–dipole interactions. Sonication of the lipid dispersion produces a sharp resonance line at frequency ν_i (cf. also ref 4, Figure 5). Also shown in Figure 1 are theoretical spectra which were calculated using the following assumptions. Let us denote with σ_{kk} ($k = 1, 2, 3$) the principal components of the static chemical shift tensor of the phosphorus nucleus in the choline group. In solution, due to the rapid tumbling of the molecule, only the average value σ_i (corresponding resonance frequency ν_i) is observed

$$\sigma_i = \tfrac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33}) \quad (1)$$

The motion of the phosphate group in a lipid bilayer is however

anisotropic and the observable chemical shift is given by

$$\sigma = \sigma_i + \tfrac{2}{3}\sigma_a\left(\frac{3\cos^2\Theta - 1}{2}\right) \quad (2)$$

with

$$\sigma_a = (\sigma_{11}S_{11} + \sigma_{22}S_{22} + \sigma_{33}S_{33}) = (\sigma_{11} - \sigma_{22})S_{11} + (\sigma_{33} - \sigma_{22})S_{33} \quad (3)$$

S_{kk} is the order parameter of the kth principal axis,[7] while Θ denotes the angle between the magnetic field and the bilayer normal. Only two order parameters are independent, the third is determined by $\Sigma^3_{k=1}S_{kk} = 0$. From eq 2 the resonance frequency is found to be[8]

$$\nu = \nu_i - \nu_a\frac{3\cos^2\Theta - 1}{2} \quad (4)$$

with $\nu_a = \frac{2}{3}\sigma_a\nu_0$ (ν_0 is the instrumental phosphorus frequency). Equation 4 gives the resonance position for a planar oriented bilayer system inclined at an angle Θ with respect to the magnetic field H_0. If the bilayers are distributed at random the shape of the resulting "powder-type" spectrum is the sum of the resonances of all possible orientations Θ. Let us define the probability function $p(\nu)$ so that $p(\nu)\,d\nu$ describes the fraction of spins with resonance frequencies between ν and $\nu + d\nu$. $p(\nu)$ is found to be[6]

$$p(\nu) \propto \left\{1 - \frac{2(\nu - \nu_i)}{\nu_a}\right\}^{-1/2} \quad (5)$$

To each resonance line ν there corresponds a transition probability $I(\nu)$. The shape of an individual resonance line centered at frequency ν^* can be appproximated by a normalized Gaussian of the form

$$I(\nu - \nu^*) = \frac{1}{\sqrt{2\pi}}\frac{1}{\Delta}\exp\{-(\nu - \nu^*)^2/2\Delta^2\} \quad (6)$$

The line width at half-height is given by

$$(\delta\nu)_{1/2} = 2.36\Delta \quad (7)$$

Now we assume that Δ is composed of a constant and an angular dependent part

$$\Delta = \Delta_0 + \Delta_1\frac{3\cos^2\Theta - 1}{2} \quad (8)$$

The orientation-dependent term describes the contribution of *intra*molecular dipole–dipole interactions, and the constant term Δ_0 is the residual line width after suppression of the intramolecular dipole–dipole interactions and arises from intermolecular dipole–dipole interactions, from the modulation of the chemical shift anisotropy, and from other relaxation mechanisms. In a powder-type spectrum the total absorption intensity $S(\nu)$ at a frequency ν is the sum over all overlapping resonances ν^* weighted with their corresponding probability $p(\nu^*)$

$$S(\nu) = \int_{-\infty}^{+\infty} I(\nu - \nu^*)p(\nu^*)\,d\nu^* \quad (9)$$

In our model the shape of the spectrum is therefore determined by three parameters, namely the chemical shift anisotropy σ_a and the line width parameters Δ_0 and Δ_1. For completely decoupled spectra this set is reduced to two parameters, since Δ_1 should become zero. The dashed curves in Figure 1 were calculated with these assumptions and the parameters for the best fit of the experimental curves are listed in Table I. It should be pointed out that the assumption of complete dipole–dipole decoupling is an oversimplification. With our available decoupling power (~20 W) the proton–phosphorus decoupling, especially at temperatures below the phase transition, is not perfect. The agreement between the experimental and computed spectra in the decoupled case can probably be improved by introducing some small Δ_1 terms.

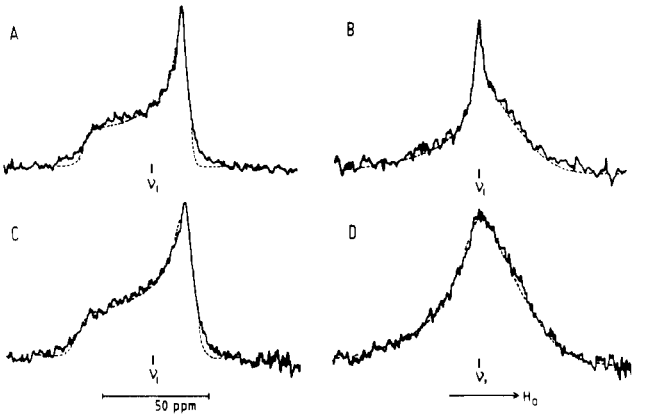

Figure 1. Phosphorus-31 NMR spectra at 36.4 MHz of unsonicated bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. (A) Proton decoupled spectrum at 44 °C, (B) nondecoupled spectrum at 44 °C, (C) proton decoupled spectrum at 38 °C, (D) nondecoupled spectrum at 38 °C. Solid line: experimental spectrum. Dashed line: theoretical spectrum calculated with the parameters given in Table I.

Table I. Spectral Parameters of Phosphorus-31 NMR Spectra of Lipid Bilayers

		Line width parameters, Hz			
		Proton decoupled spectra		Nondecoupled spectra	
Temp, °C	Chemical shift anisotropy σ_a, ppm	Δ_0	Δ_1	Δ_0	Δ_1
29	−58.6	300	0	350	800
38	−53.5	125	0	200	800
44	−49.4	70	0	20	1000
59	−44.3	70	0	5	1000
73	−42.2	70	0	5	1000

Discussion

Inspection of Table I reveals that Δ_0 is the parameter with the strongest temperature dependence. This can be explained by a contribution to the line width from temperature-dependent *inter*molecular dipole–dipole interactions. At high temperatures the lateral diffusion is rapid enough to average most of the intermolecular interactions; at low temperatures the mobility of the lipid molecules is reduced and intermolecular effects become increasingly important. In our model the line width parameter Δ_0 is expected to have the same value for decoupled and nondecoupled spectra above the phase transition. This is only approximately borne out by the computer simulation. The reason for this discrepancy is the above mentioned incomplete decoupling.

Under nondecoupled conditions the shape of the phosphorus spectrum is dominated by Δ_1. The essential feature is the sharpening of the lines close to the magic angle which explains the intense peak at frequency ν_i observed for nondecoupled spectra.

The shape of the decoupled phosphorus spectra, on the other hand, is governed by the chemical shift anisotropy σ_a. The edges of the spectra correspond to the orientations $\Theta = 0$ and 90° and the separation of the edges is approximately σ_a. The computer simulation of the spectra allows a rather precise determination of σ_a and this residual chemical shift anisotropy may then be compared with recent results of Kohler and Klein for the static chemical shift tensor of dipalmitoyl-3-*sn*-phos-

phatidylcholine. Using their data eq 3 takes the form

$$\sigma_a = -56S_{11} + 133S_{33} \ (\sigma_a \text{ in ppm}) \qquad (10)$$

S_{11} is the order parameter of the principal axis connecting the esterified oxygens, S_{33} refers to the connecting axis of the other two oxygens on the phosphorus. Unfortunately the static chemical shift tensor of dipalmitoyl-3-*sn*-phosphatidylcholine is not axially symmetric and the motion of the phosphate group must be described by *two* order parameters. Without any additional information a quantitative interpretation of the σ_a values is thus impossible. However, a qualitative conclusion can be reached on the basis of previous deuterium NMR results obtained for the same system.[4] In the light of these experiments it is safe to assume that neither S_{11} nor S_{33} are larger than +0.5. Since σ_a is approximately −50 ppm it follows from eq 10 that S_{33} must be negative. Therefore the connecting axis of the nonesterified oxygen atoms must be oriented preferentially perpendicular to the bilayer normal.

Further insight into the motion and orientation of the phosphate group should be obtained by phosphorus NMR measurements with partially deuterated phospholipids. Upon proton decoupling the line width parameter Δ_1 should be determined exclusively by the remaining deuteron–phosphorus dipole–dipole couplings. The well-defined deuteron–phosphorus couplings of selectively deuterated lipids could provide the missing link for the quantitative analysis of σ_a in terms of eq 10.

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