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Observation of the Effect of Water on the ^{31}P Nuclear Magnetic Resonance Spectra of Dipalmitoyllecithin

Sir:

Phospholipids, one of the primary components of cell membranes, have been extensively examined with a variety of physical and spectroscopic techniques. However, the motion of the polar head group of phospholipids and the factors which affect this motion are as yet poorly understood. In addition little direct information is available on the specific orientation of the phosphate or choline moieties in a phospholipid bilayer. In order to investigate these phenomena, we have begun an examination of the ^{31}P NMR spectra of phospholipids and we present a preliminary account of our results here; specifically, we have studied dipalmitoyllecithin (DPL) at varying water concentrations. Initially we chose a relatively low temperature (15 °C) and low water content (0-10%) because we wished to observe a nearly rigid lattice ^{31}P chemical shift anisotropy. However, we
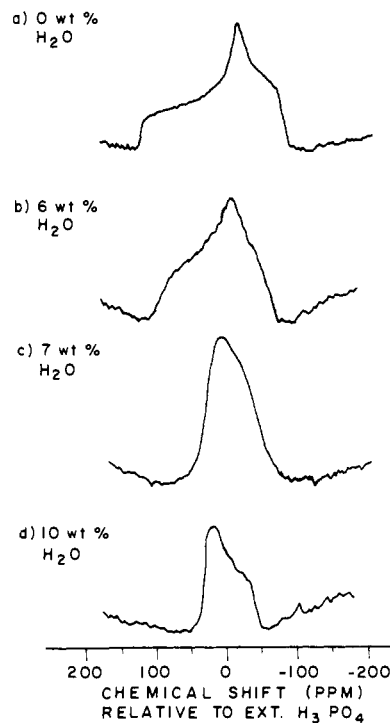

Figure 1. Proton decoupled ^{31}P spectra of DPL as a function of water concentration at 15 °C. $\nu_{31P} = 118.5$ MHz.

Table I. ^{31}P Chemical Shift Tensor Components of DPL as a Function of Water Concentration at 15 °C Relative to External 85% H_3PO_4. Errors in the Principal Values are Discussed in the Text

Wt % H_2O	σ_{11}	σ_{22}	σ_{33}	$1/3 \Sigma(\sigma_{ii})$
0	−97.6	−34.5	131.0	−0.4
6	−68.9	−18.1	96.9	3.3
7	−54.7	20.2	20.2	−4.8
10	−38.9	21.4	21.4	1.3

have found that under these conditions the spectra reveal interesting changes in what we think are the motional properties of the phosphate, and these changes allow us to draw conclusions regarding the effect of water on the conformation of the phosphate part of the polar head group.

Figure 1a shows a proton decoupled ^{31}P powder spectrum of anhydrous DPL[1] which is ~230 ppm wide, and we discern from the spectrum that the ^{31}P shift tensor is axially asymmetric. The principal values of the shift tensor, relative to external 85% H_3PO_4, are given in Table I. Upon addition of 6, 7, and 10 wt % H_2O, Figures 1b–d, we find that the broad spectrum of Figure 1a collapses to an axially symmetric pattern of ~60 ppm breadth and of reversed sign. Increasing the water concentration further (we have examined 12, 22 and 46 wt %) produces no additional change in the breadth or shape of the powder spectrum at this temperature. The principal values of the shift tensor components at each water concentration are also compiled in Table I where we have used the convention $\sigma_{11} < \sigma_{22} < \sigma_{33}$. The errors in the principal values are ±0.5 ppm for spectra 1a and 1d while for spectra 1b and 1c they are ±1.5 ppm. The larger errors for the latter spectra are due to the fact that the shoulders which determine the principal values are, as one can see, not well defined. The spectra of Figure 1 are intended to illustrate the collapse of the ^{31}P powder pattern, and a precise knowledge of the principal values of the shift tensors, or of the sample water contents at intermediate water concentrations, is not crucial to the conclusions which we discuss below.

We believe that the water induced narrowing and subsequent sign reversal of the ^{31}P powder spectrum of DPL is due to the onset of rotation of the PO_4 group. It has been shown that rapid anisotropic motion about an arbitrary axis will reduce an axially asymmetric powder spectrum to an axially symmetric spectrum[2] as we observe with DPL. Furthermore, the trace of a shift tensor is invariant to anisotropic motion and one can discern from Table I that the traces of the ^{31}P tensors which we observe for DPL at various water concentrations are identical within experimental error. An alternative explanation for the narrowing of the spectrum is that water is hydrogen bonding to the phosphate and consequently perturbing the tensor elements; for instance, it is known that protonation does alter ^{31}P chemical shifts[3a] and protonation effects have been observed in ^{13}C spectra of carboxyl shift tensors.[3b] However, it is difficult to estimate the size or direction of the shifts that will be produced by these effects since there have been no studies of the anisotropy of phosphate shielding tensors as a function of hydrogen bonding. Nevertheless, if we assume that hydrogen bonding could alter the shift tensor components, then it seems unlikely that it would result in an axially symmetric tensor and that the trace would remain unchanged. Thus, although we cannot completely exclude this latter alternative, we believe the most plausible explanation for the effect we observe is motional averaging. We plan further experiments to clarify this point.

The results we report are in agreement with conclusions obtained from proton NMR studies of DPL at similar temperatures and water concentrations. Veksli et al.[4] observed a narrowing of the broadline proton spectrum of DPL at 24 °C which was complete at 4–5 mol of H_2O per mole of lipid. On the basis of measurements of relative intensities of lines in their spectra, these workers assigned one of the lines to the choline protons and interpreted the narrowing of this line as due to onset of motion of the polar head. Furthermore, Tardieu et al.,[5] on the basis of x-ray studies of lecithin with saturated fatty acid chains, reported a phase boundary in the neighborhood of 5 wt % water for dimyristoyllecithin; this phase boundary is also present in the DPL–water system but is shifted to slightly higher water concentrations[6] in agreement with our results.

In addition, our data may be of use in understanding the nature of the calorimetric pretransition observed at 34 °C in differential scanning calorimetry experiments.[7] This transition has been associated with motion of the choline group,[8] but our data, together with those of Gally et al.,[9] indicate that this may not be a completely correct interpretation. Gally et al. have shown that at 30 °C the ^{31}P spectrum of DPL in 50 wt % H_2O exhibits an axially symmetric powder pattern of 59 ppm breadth[10] which narrows slightly to 53 ppm at 40 °C. The breadth at 30 °C is identical with what we find at ~10 wt % H_2O and 15 °C and, thus, raising the temperature of the sample and adding additional water apparently have little effect upon the motion of the phosphate—the motional transition of the PO_4 is complete at much lower temperatures and water concentrations than are necessary for the observation of the pretransition. Thus, while there is some chemical evidence linking the pretransition to the head group, e.g., ions which bind to the PO_4 remove it,[8] to say it involves *motion* of the head group may be an oversimplification. We plan to explore this point further in the near future.

The fact that the ^{31}P powder spectrum of DPL is relatively invariant to temperature and water above 15 °C and 10 wt % leads to another interesting conclusion. As we mentioned above one can show by straight forward arguments that rapid anisotropic rotation about an arbitrary axis will reduce an axially asymmetric powder spectrum to an axial-

ly symmetric spectrum.[2] Furthermore, the breadth of this spectrum is determined by the eigenvalues of the rigid lattice shift tensor and by the orientation of the tensor relative to the axis of rotation. Thus, if one knows the orientation of the rotation axis in the molecule and the rigid lattice tensor values, then by measuring the breadth of the motionally averaged tensor one can calculate the orientation of the rigid lattice tensor in the molecular frame. Such a technique was used to determine the orientation of ^{19}F shielding tensors in a rotating CF_3.[2] It should be clear from the above considerations that the breadth of the rotationally averaged shift tensor will change further only if the orientation of the shift tensor relative to the rotation axis is altered, e.g., if there is a conformation change. From these considerations and the invariance of the breadth of the ^{31}P spectrum to water content above 10 wt % we reach the following important conclusion: *for DPL at 15 °C the conformation at the PO_4 is determined by the first 4–5 waters of hydration and this conformation is not significantly affected by additional water.* Moreover, the fact that Gally et al. observe a ~60 ppm breadth in the 30–40 °C range implies little change in conformation compared to that found at 15 °C. One would like to extend this conclusion to the remainder of the head group; however, this is probably not justified since there are other molecular degrees of freedom available. Prior to our experiments there were other data in the literature, for instance Chapman's broadline study,[7] which hinted at the importance of the first few waters of hydration in determining head group conformation. However, these data were not of a definitive nature and thus the conclusion at which we arrive is new.

It has recently been shown that observation of powder spectra as a function of temperature will allow one to distinguish between discrete and continuous rotational diffusion; in solid P_4 the phosphorus tetrahedra reorient by 109° rotations[11] and in hexamethylbenzene[12] the benzene-rings reorient by 60° jumps about the molecular C_6 axis. We plan to examine the temperature and H_2O concentration dependence of phospholipid spectra in order to study the type of motion the phosphate is executing. In addition, if either the orientation of the ^{31}P shift tensor in the molecular frame or the axis of rotation is known, then one can calculate the other.[2] For this reason we and others[13] are examining single crystal spectra of phosphate containing compounds in order to determine the orientation of chemical shift tensors in the molecular frame. When these data are available they should allow a determination of the axis of rotation of the phosphate relative to the molecular frame, and, with data from oriented systems, the orientation of the phosphate in a lipid bilayer.

These experiments were performed on a homebuilt pulse spectrometer operating at a ^{31}P frequency of 118.5 MHz.[14] A rotating field of ~10 G was employed for proton decoupling. The pulse sequences used in collecting the data are described elsewhere.[15]

References and Notes

(1) Anhydrous DPL was obtained by heating the lipid obtained from Calbiochem for 4 h at 90 °C under vacuum and sealing it without exposure to the atmosphere.[7] The low water content samples (≤12 wt %) were prepared by first weighing ~100 mg of the monohydrate form of the

lipid and then monitoring the increase in weight upon exposure to a humid atmosphere until the desired water content was obtained. For the high water content samples the appropriate quantity of water was pipetted into a vial containing the lipid. Homogenization of the low water-samples was accomplished by heating the sealed samples to above the gel-liquid crystalline transition[9] and for the high water samples (22 and 46 wt %) the lipid-water mixtures were repeatedly forced through a constriction. We believe the water contents are accurate only to ±1%, and thus the 1% difference between Figures 1b and 1c should not be taken too seriously.

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Figure 1. Energy dependence of the relative total cross sections for the reactions: (O) $F_2 + I_2$; (●) $F_2 + ICl$; (△) $F_2 + HI$.

Figure 2. Schematic diagram of the reaction energetics for $F_2 + I_2$.

Iodine–Fluorine Bond Strength in IIF, ClIF, and HIF

Sir:

There has long been speculation regarding the existence of triatomic halogen molecules.[1] These molecules have often been postulated as intermediates in photochemical reactions.[2-4] From the temperature dependence of the recombination rate of I atoms in the presence of I_2 the stability of I_3 has been estimated to be about 5 kcal/mol.[4] However, no triatomic halogen radical species has been detected in the gas phase, although Cl_3 has been identified from its infrared spectrum in a matrix isolation experiment.[5]

We wish to report here the direct observation of the trihalogens IIF and ClIF and the pseudo-trihalogen HIF resulting from an endoergic, bimolecular reaction of F_2 and I_2, ICl, or HI in a crossed molecular beam experiment. Figure 1 shows the threshold behavior of the formation of I_2F, ClIF, and HIF products as the relative kinetic energy between F_2 and I_2, ICl, or HI is varied. From the observed threshold and the dissociation energy of F_2 (36.7 kcal/mol)[6] lower bounds on the I–F bond strength in I_2F, ClIF, and HIF can be established as 33, 31, and 26 kcal/mol, respectively. These bond energies are comparable to the IF bond strength of 25 kcal/mol in the recently observed CH_3IF,[7] and are approximately one-half to one-third of the IF dissociation energy of 66.4 kcal/mol.[8] Figure 2 shows a schematic diagram of the reaction energetics for one of the systems, $F_2 + I_2$. Similar diagrams can be constructed for the $F_2 + ICl$ and $F_2 + HI$ systems.

The apparatus used here has been described previously.[9] It employs two crossed supersonic beams of reactants. The products of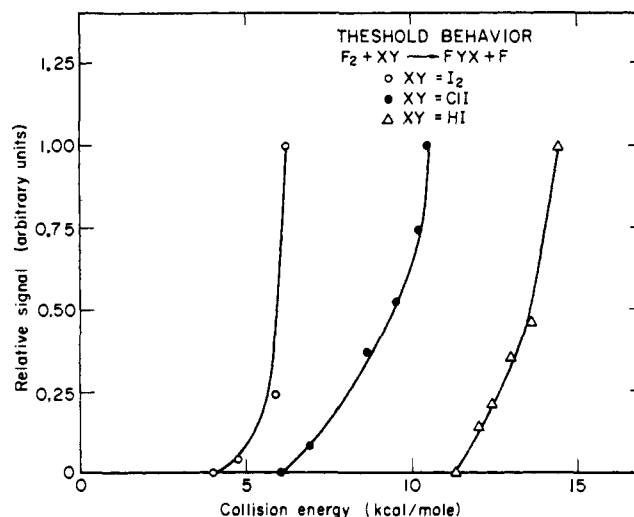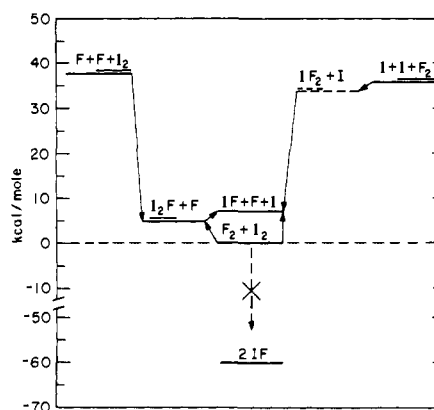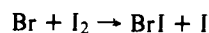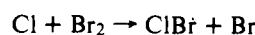 the reaction are detected in the plane of the reactant beams by a rotatable, triply differentially pumped mass spectrometer detector utilizing electron bombardment ionization and ion counting. In these experiments a beam of pure HI or a beam of I_2 or ICl in argon carrier gas is expanded from a glass nozzle. This halogen beam is crossed with a hyperthermal beam of fluorine molecules seeded in helium (or a helium-neon mixture). This fluorine beam is produced by expansion from a resistance heated nickel nozzle. By varying the relative abundances of the fluorine and the inert carrier gases, and the temperature of the nozzle, the relative collision energy has been varied continuously from about 3 kcal/mol to more than 30 kcal/mol.

The existence of these interhalogen compounds is not surprising in view of recent crossed molecular beam experiments involving halogen containing molecules. CH_3IF has been observed in an experiment using beams of F_2 and CH_3I.[7] Halogen atom–halogen molecule reactions have shown evidence of short-range attraction. Reactive scattering in systems such as:

$$Cl + I_2 \rightarrow ClI + I$$

$$Cl + IBr \rightarrow ClI + Br$$

$$Cl + BrI \rightarrow ClBr + I$$

$$Cl + Br_2 \rightarrow ClBr + Br$$

$$Br + I_2 \rightarrow BrI + I$$
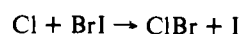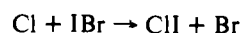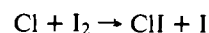
gives product angular distributions which can be well accounted for by an osculating complex model involving tri-