the two equations becomes apparent.

We express the log of the solubility as a function of temperature by means of eq 12. At T_0 where $\Delta H^\circ = 0$, the natural log of the solubility is

$$\ln \mathrm{sol}_0 = \frac{\Delta H^\circ_0}{RT_0} + \frac{\Delta C_p}{R} \ln T_0 + \frac{(\Delta S^\circ_0 - \Delta C_p)}{R} \quad (13)$$

Subtracting eq 13 from eq 12, expanding the right-hand term in a power series, and neglecting the terms above those in T^2, one obtains

$$\ln \mathrm{sol} - \ln \mathrm{sol}_0 = -\frac{\Delta H^\circ_0}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right) - \frac{\Delta C_p}{R}\left[\left(\frac{T_0}{T} - 1\right) - \frac{1}{2}\left(\frac{T_0}{T} - 1\right)^2\right] \quad (14)$$

Keeping in mind that $-\Delta H^\circ_0/R = \Delta C_p T_0/R$, eq 14 can be rewritten as

$$\ln \mathrm{sol} = \frac{\Delta C_p T_0^2}{2R}\left(\frac{1}{T^2}\right) - \frac{\Delta C_p T_0}{R}\left(\frac{1}{T}\right) + \left(\frac{\Delta C_p}{2R} + \ln \mathrm{sol}_0\right) \quad (15)$$

Since ΔC_p, T_0, R, and $\ln \mathrm{sol}_0$ are all temperature independent or assumed to be so. Equation 15 is equivalent to eq 7 as used by Ueda et al.[8,18] where their constants A, B, and C are represented in the following way:

$$A = \Delta C_p T_0^2/2R$$

$$B = -\Delta C_p T_0/R$$

$$C = \Delta C_p/2R + \ln \mathrm{sol}_0$$

Thus it can be seen, for example, that the temperature-independent term C, which they suggested as representing the hypothetical solubility at some infinitely high temperature, actually contains ΔC_p, R, and the logarithm of the solubility at T_0 which falls in the experimental range.

Although the detailed theoretical treatment of aqueous solutions of hydrocarbons, particularly mixed aqueous solutions, promises to be a difficult one, the general thermodynamic laws governing the solubilization process will be useful. For the present we feel that methanol and ethanol exert their influence upon hydrocarbon solubility by bringing about a decrease in water structure and release of somewhat immobilized water molecules as the hydrophobic interaction between hydrocarbon and alcohol takes place in the aqueous medium.

Acknowledgment. The authors are indebted to Dr. Yoichi Takabayashi of this department for translation of ref 8 from the Japanese. The authors also wish to thank Drs. Gunter Franz and Millecchia of the Department of Physiology and Biophysics for their helpful comments and discussion.

Registry No. Naphthalene, 91-20-3; ethanol, 64-17-5; water, 7732-18-5.

Behavior of Hexane Dissolved in Dimyristoylphosphatidylcholine Bilayers: An NMR and Calorimetric Study

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Abstract: Deuterium and phosphorous NMR spectroscopy and differential scanning calorimetry (DSC) have been used to examine the behavior of dimyristoylphosphatidylcholine (DMPC) bilayers to which hexane has been added. This work represents a characterization of a two-component bilayer system in which the minor component is a simple example drawn from the large class of hydrophobic molecules that are located in the biological membrane or can be incorporated into it. DSC thermograms indicate that the phase behavior of this mixture is similar to bilayers composed of a mixture of dilauryolphosphatidylcholine and distearoylphosphatidylcholine. Isothermal melting is observed at about 0 °C. Deuterium NMR spectra of perdeuterated hexane dissolved in the bilayer show three overlapping powder patterns indicating that on average both ends of the molecule are experiencing the same environment. Deuterium NMR spectra of macroscopically oriented bilayers indicate that the direction of motional averaging is the same for both the lipids and hexane, i.e., normal to the bilayer plane. The temperature dependence of the hexane–deuterium quadrupole splittings exhibits a maximum at the lipid–bilayer phase-transition temperature. At about the isothermal melting temperature the powder patterns coalesce into a single line, indicating the onset of isotropic motion. Deuterium NMR spectra of acyl-chain perdeuterated DMPC and phosphorus NMR spectra of the DMPC head group change little upon the addition of hexane. Taken together these results indicate that at hexane to lipid ratios of less than about 0.5 the bilayer is intact and the order of the DMPC molecules is little affected by the presence of the alkane. The hexane motion giving rise to three powder patterns is, no doubt, quite complex, but we envision it as encompassing a shuffling between the two monolayers in a direction normal to the bilayer surface as well as rotation about this normal axis and gauche–trans isomerization.

Introduction

The primary structural element of the biological membrane is the lipid bilayer. The interior of the bilayer is a mixture of the alkyl chains of the amphiphilic molecules of which the bilayer is composed. The partition of molecules between the bilayer and the surrounding aqueous phase plays a major role in determining the structure and function of the biological membrane. The most widely accepted architectural model for the membrane is the fluid mosaic model in which integral protein "solute molecules" are dispersed (or aggregated) within the two-dimensional bilayer "matrix" or "solvent".[1] Thus, the interior of the membrane is often taken to be an alkyl solvent in which other molecules are

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0002-7863/84/1506-0915$01.50/0

dissolved. This hypothesis has proved useful in studying the mechanism of general anesthetics[2,3] and carrier-mediated ion transport.[4] The characterization of this quasi two-dimensional system in terms of solvent–solvent, solvent–solute, and solute–solute interactions is a complex problem. The difficulty is due principally to the multiplicity of kinds of lipids that make up the "solvent" and the complexity of the protein "solute" molecules. As a first step toward understanding such a system, we have chosen to study single-component lipid bilayers to which have been added simple hydrophobic solutes. In the present case the "solute" is a simple hydrophobic molecule, hexane. The "solvent" is dimyristoylphosphatidylcholine (DMPC) in excess water.

Besides being well-defined model systems, the alkane/lipid bilayer solutions are used extensively in black lipid membrane studies[5,6] and are of interest for physiological reasons; e.g., the shorter chain alkanes are anesthetics.[7] We chose to study hexane in DMPC because of the convenient phase transition at 23 °C and the existence of thermodynamic studies by Simon and colleagues.[8,9] We have employed differential scanning calorimetry (DSC) to study the thermodynamics of DMPC/hexane bilayer mixtures and deuterium and phosphorous NMR to study the microscopic behavior of this system. We have limited our examinations to mixtures of alkane, lipid, and water with compositions giving only lamellar phases. Thus the lipid ^{31}P NMR spectra show little change with either temperature or hexane concentration, despite significant changes in the DSC thermograms induced by even very small amounts of hexane. The ^2H NMR spectral characteristics of ^2H-labeled hexane dissolved in the bilayer are strong functions of both hexane concentration and temperature. The ^2H NMR spectra of ^2H-labeled DMPC are altered only slightly by the introduction of hexane.

Experimental Procedure

Materials. Lipids (both ^2H labeled and unlabeled) were purchased from Avanti Polar-Lipids, Inc. (Birmingham, AL), deuterium-depleted water (^2H content = 1% of natural abundance) from Sigma (St. Louis, MO), hexane (pesticide grade) from Burdick and Jackson Laboratories, Inc. (Muskegon, MI), and perdeuterated hexane from KOR Isotopes, Inc. (Cambridge, MA). Tritiated hexane and methyl-deuterated hexane were synthesized from 1-bromohexane via reduction with $LiAl^3H_4$ and $LiAl^2H_4$. All alkanes used were checked for purity by using gas chromatography and found to be greater than 99% pure. Thin-layer chromatography was used to check lipid purity.

Sample Preparation. A series of mixtures of DMPC, hexane, and ^2H-depleted water were prepared. In all cases approximately 100 mg of lipid was dried from chloroform/methanol (2:1 volume ratio) to form a thin film on the inside of a 25-mL round-bottom flask. A hexane/hexadecane mixture of defined composition was placed in another flask, and ^2H-depleted water was put in a third. All three flasks were connected, the temperature was controlled at 27 °C, and the water and alkane flasks were stirred. After 12 h, 0.3 mL of ^2H-depleted water was added to the lipid flask via a septum. The lipid/alkane/water mixture was warmed and gently vortexed to thoroughly disperse the lipid. The mixture was then withdrawn and placed in the NMR sample tube (7 mm diameter, 12–15 mm long) which was then sealed. In all cases the hexane (unlabeled or ^2H labeled) was doped with a small amount of tritiated hexane. The radioactivity of an aliquot of the final sample allowed the amount of hexane in the aliquot to be assayed. A phosphate assay[10] was used to determine the amount of lipid in the same aliquot. The mole percent of hexane was then calculated as the moles of hexane divided by the total number of moles of lipid and hexane. The uncertainty in the mole percents of hexane noted below is 2 mol %. A similar procedure was employed for the macroscopically oriented samples, except that the lipid was initially deposited on 8–10 microscope cover slips. After evaporation of solvent and equilibration in the hexane and water atmosphere, the cover slips were stacked and approximately 10 μL of water was drawn up between the slips via capillary action. The stack of slips was then inserted into the NMR sample tube, which was then sealed.

NMR Spectroscopy. ^2H NMR spectra were obtained at the NSF Southern California Regional NMR Center on a modified Bruker WM-500 spectrometer in the Fourier transform mode at 76.8 MHz (magnetic field strength of 11.7 T). All ^2H spectra were taken by using the quadrupole echo technique[11,12] with τ_{echo} = 40 μs, and a 90° pulse of 6–9 μs. The pulse repetition rate was 1 s. The number of scans per spectrum varied from 1000 to 10000. All free-induction decays were processed with an exponential multiplication factor of 40 Hz. Quadrature phase detection and full proton decoupling conditions were employed. The data acquisition rate was 166 666 Hz. T_{2echo} values were determined by measuring peak heights as a function of τ_{echo}. No change in the spectral line shapes were observed as τ_{echo} was changed. Heights were then corrected for the intensity of overlapping spectral features. Log-linear plots of corrected peak heights vs. $2\tau_{echo}$ yielded straight lines whose slope was $-1/T_{2echo}$. The estimated uncertainty in T_{2echo} is 15%. Sample temperature was controlled with a flow of temperature-regulated nitrogen gas. In all cases the sample was allowed to reach thermal equilibrium before data acquisition was begun. Typically, this took 20 min after the probe temperature had stabilized at the desired temperature.

Phosphorus-31 NMR spectra were obtained on a Bruker WM-250 spectrometer (Chemistry Department, UCI) at 101.3 MHz (magnetic field strength of 5.9 T) operating in the Fourier transform mode. The 90° pulse was 30 μs. Approximately 10 W of gated proton decoupling was employed. An exponential multiplication factor of 100 Hz was employed. The data acquisition rate was 50 kHz. The pulse repetition rate was 1 s. Under these conditions there is significant pulse power falloff across the spectrum and incomplete proton decoupling. This results in a somewhat distorted line shape, but changes in the chemical shift anisotropy ($\Delta\sigma$) from one spectrum to another can be measured accurately. Temperature control was accomplished in the same manner as described for the ^2H NMR experiments.

Calorimetry. The differential scanning calorimetry was performed on a Perkin-Elmer DSC-2B with a liquid nitrogen cooling accessory. In all cases aliquots were taken from the NMR samples and immediately sealed in aluminum DSC pans. A typical DSC sample contained about 2 mg of DMPC/hexane and 5 mg of water. For each thermogram the sample was cooled to −13 °C, held there for 15–30 min, are then heated to 32 °C at 1.25 °C/min. With this protocol no freezing of the water in the sample was observed. Subsequent scans of the same sample exhibited a slight broadening of the endotherms, but were qualitatively the same as the initial run. Low-temperature thermograms were run in essentially the same manner except that the temperature span was from −113 to −83 °C. Heat absorption onset and completion temperatures were determined as described by Mabrey and Sturtevant[13] except that no correction was made for the finite width of the pure DMPC-bilayer phase transition.

Theoretical Background

Derivations and discussions of solid-state ^2H and ^{31}P NMR in terms of Hamiltonians, order parameters, and "powder pattern" line shapes can be found elsewhere.[14–18] In ^2H NMR spectra of labeled lipids dispersed as multilamellar structures in water, one observes a powder pattern. In this powder pattern the separation between peak maxima ($\Delta\nu_q$: the quadrupole splitting) is related to the C–^2H order parameter (S_{CD}) by

$$\Delta\nu_q = \frac{3}{4}\frac{e^2qQ}{h}S_{CD}$$

where e^2qQ/h is the static quadrupole coupling constant (167 kHz for the C–^2H bond).[19,20] In a multiply labeled molecule (alkane

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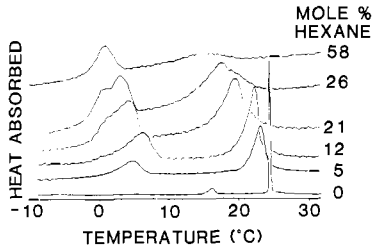

Figure 1. Thermograms of aliquots of the hexane/DMPC/H_2O samples used in the NMR experiments. Heating rate was 1.25 °C/min. The vertical scale is not the same for each trace.

Table I. Transition Enthalpies for Hexane/DMPC/Water Mixtures

mol % hexane	enthalpy[a] full temperature range	enthalpy[a] high temperature peak
0	7.6	6.3
5	12.1	7.1
13	12.2	6.3
21	13.6	
26	13.1	
58	14.0	

[a] In units of kcal/mol DMPC. Uncertainty is approximately 0.5 kcal/mol DMPC.

or lipid) there will be a powder pattern for each distinct deuterium, thus an order parameter for each distinct C–^{2}H bond.[21]

Proton-decoupled ^{31}P NMR spectra of multilamellar lipids containing phosphatidylcholine headgroups are characterized by an axially symmetric ^{31}P powder pattern with $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp} = -40$ to -50 ppm.[15] In such a situation $\Delta\sigma$ is proportional to the order parameter for the phosphate group. In these spectra $\sigma_{\parallel}$ is located at the upfield peak and $\sigma_{\perp}$ is located at the downfield shoulder. Due to experimental difficulties (vide supra), only qualitative changes in $\Delta\sigma$ and the powder pattern line shape will be considered below.

Results

Calorimetry. As expected, the introduction of hexane into the bilayer causes the lipid gel-to-liquid crystalline phase transition to broaden and shift to lower temperatures (see Figure 1). The ΔH for this transition is essentially the same in the 0, 5, and 13 mol % hexane mixtures (see Table I). There is a second heat absorption event at a lower temperature. Above 13 mol % hexane, the separation of the two events is an equivocal process. The total heat absorbed per mole of lipid over the entire temperature range does not change appreciably on going from 5 to 26 mol % hexane. Qualitatively similar thermograms have been obtained with dipalmitoylphosphatidylcholine/hexane mixtures.[9] Only at the highest hexane concentration examined was any heat absorption observed in the −113 to −83 °C range. In the 58 mol % hexane mixture a small endotherm was observed at −97 °C. If attributed to pure hexane, the heat absorbed would account for less than 5% of the hexane present. A plot of the onset and completion temperatures for the endotherms is shown in Figure 2. The essentially horizontal straight line formed by the onset temperatures indicates that the mixture undergoes isothermal melting at about 0 °C.

Phosphorus NMR. The ^{31}P NMR spectra of pure diacylphosphatidylcholine dispersions are relatively insensitive to temperature changes.[15] They possess the line shape anticipated for a powder spectra with an axially symmetric chemical shift anisotropy where $\Delta\sigma = -50$ ppm. The intrinsic line width does increase as the temperature is lowered through the lipid-phase transition and $\Delta\sigma$ increases.[22] Figure 3 shows ^{31}P NMR spectra

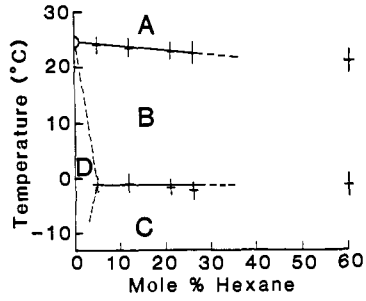

Figure 2. Onset and completion temperatures derived from the thermograms in Figure 1.

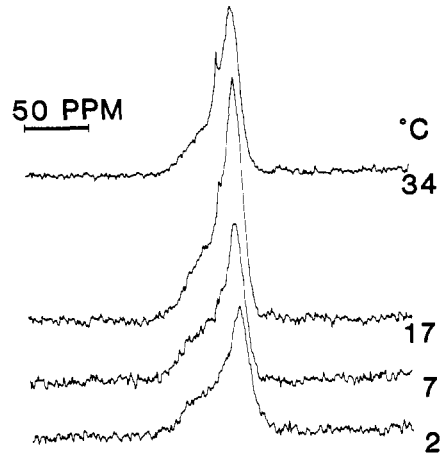

Figure 3. ^{31}P NMR spectra of DMPC multilayers with 26 mol % hexane at various temperatures.

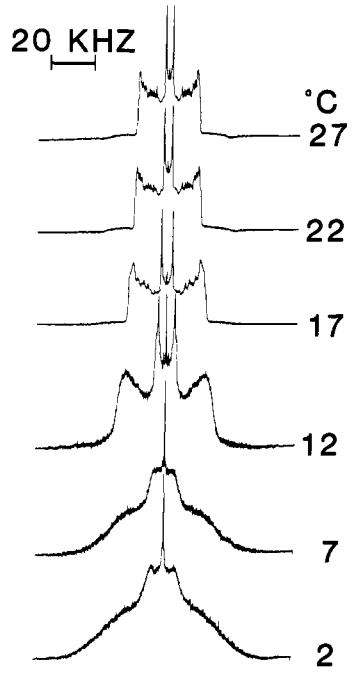

Figure 4. ^{2}H NMR spectra of acyl-chain perdeuterated DMPC multilayers with 26 mol % hexane at various temperatures. The central peak is actually slightly off center and its absolute intensity does not change with temperature. Therefore, we attribute it to residual HOD.

that are representative of all the DMPC/hexane samples used in the DSC experiments. The intrinsic line width increases slightly as the temperature is lowered. The pure DMPC spectra (data not shown) and 26 mol % hexane spectra are superimposable.

Deuterium NMR. The temperature dependence of the ^{2}H NMR spectrum of DMPC with both acyl chains perdeuterated (per-^{2}H-DMPC) plus 26 mol % hexane is shown in Figure 4.

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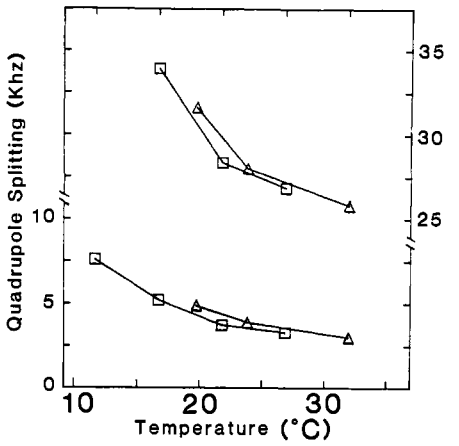

Figure 5. Graph of the innermost (lower trace) and outermost (upper trace) quadrupole splittings from ^2H NMR spectra of acyl-chain perdeuterated DMPC with 26 mol % hexane (□) and without hexane (△) as a function of temperature.

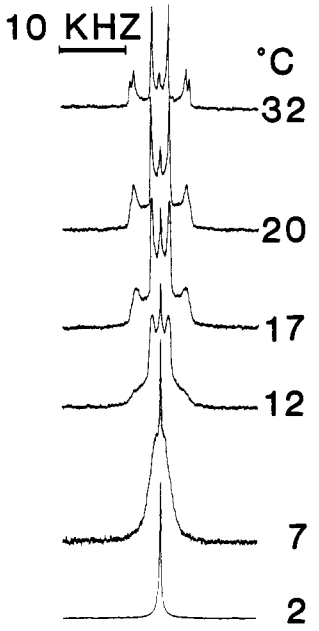

Figure 6. ^2H NMR spectra of 3 mol % perdeuterated hexane in DMPC multilayers at various temperatures.

Figure 5 shows the temperature dependence of the methyl quadrupole splitting (innermost powder pattern) and the outermost quadrupole splitting. The general appearance of the spectra and the temperature dependence of the $\Delta\nu_q$'s are similar to those found in pure per-^2H-DMPC bilayers.[21]

In contrast with the lipid NMR data, the ^2H NMR spectrum of perdeuterated hexane (per-^2H-hexane) dissolved in the DMPC bilayer is strongly dependent upon both temperature and hexane concentration. Figures 6 and 7 show the effect of changes in temperature and composition on the per-^2H-hexane ^2H NMR spectrum. At high temperatures and low to moderate hexane concentrations the spectra are a composite of three distinct powder patterns with relative intensities of 3:2:2. When [1-$^2H_1$]hexane is employed, only the innermost powder pattern is observed. As the temperature is lowered to about 22 °C the quadrupole splittings increase (see Figure 8); i.e., the order parameter for hexane motion increases. This is the expected behavior if the dissolved hexane reflects the increasing order found in the pure lipid bilayer as temperature decreases.[23] As the temperature is decreased below 22 °C the hexane order parameters decrease (the $\Delta\nu_q$'s decrease) and the lines broaden. The magnitude of $\Delta\nu_q$ and

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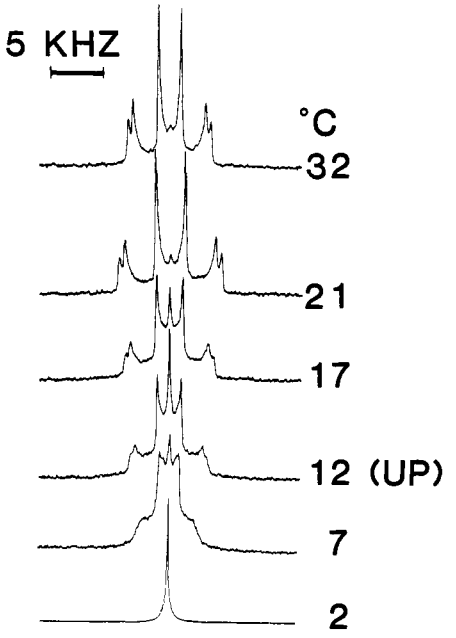

Figure 7. ^2H NMR spectra of 26 mol % perdeuterated hexane in DMPC multilayers at several temperatures. The 12 °C spectrum was recorded immediately following the 2 °C spectrum.

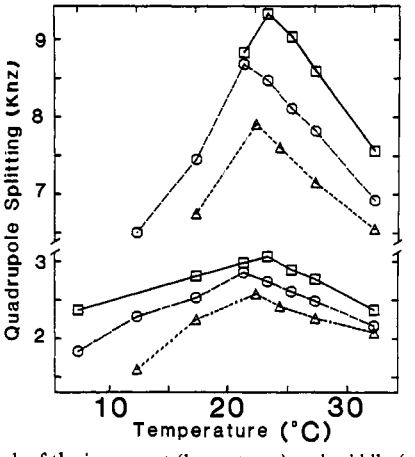

Figure 8. Graph of the innermost (lower trace) and middle (upper trace) quadrupole splittings from perdeuterated hexane in DMPC multilayers as a function of temperature at several concentrations: □, 13 mol %; ○, 21 mol %; △, 26 mol %.

Table II. $T_{2\,echo}$ Values for 26 mol % Perdeuterated Hexane in DMPC Bilayers

temp, °C	$T_{2\,echo}$, ms: isotropic component	$T_{2\,echo}$, ms: inner powder pattern
27	2.19	2.76
17	2.30	2.48
12	2.77	2.32
7	2.90	

the precise temperature at which the maximum in $\Delta\nu_q$ vs. temperature occurs both decrease as the mol % of hexane in the system increases. The temperature dependence of the T_{2echo} values for the central line and innermost powder pattern are shown in Table II.

At the low temperature extreme, regardless of hexane concentration, a single resonance is always observed—the powder pattern disappears. This effect is reversible. Raising the temperature after the powder pattern disappears causes it to reappear (see Figure 7). Above 5 °C the integrated intensity of the central line is always less than 10% of the total intensity. At very high

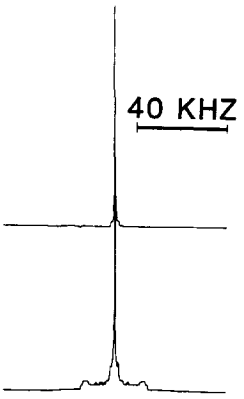

Figure 9. ^{2}H NMR spectrum of 38 mol % hexane in acyl chain perdeuterated DMPC bilayers (upper trace) and 58 mol % perdeuterated hexane in DMPC bilayers (lower trace) at 22 °C.

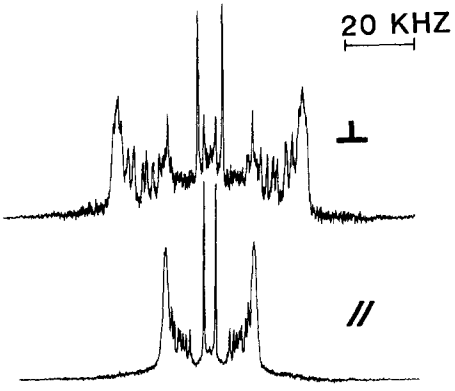

Figure 10. ^{2}H NMR spectra of macroscopically oriented multilayers of acyl-chain perdeuterated DMPC bilayers. The bilayer planes are oriented normal to the static magnetic field in the upper trace and parallel to it in the lower trace.

hexane contents the ^{2}H NMR spectra of both the lipid and alkane exhibit a significant isotropic component throughout the temperature range examined (0–30 °C). Spectra for an intermediate temperature are shown in Figure 9. As the temperature is lowered the powder pattern component broadens and disappears.

Figures 10 and 11 show ^{2}H NMR spectra of bilayer samples macroscopically oriented between glass plates. Two orientations are shown: one in which the glass plates are normal to the static magnetic field (H_0), and one in which the glass plates are parallel to H_0. Figure 10 shows the spectra of acyl chain perdeuterated DMPC at 27 °C. The observed dependence of the distance between peaks symmetric about the center of the spectrum ($\Delta\nu(\theta)$) upon the angle between the glass plates and H_0 (θ) indicates that the bilayer planes lie parallel to the glass plates; i.e., $\Delta\nu(\theta) \propto 3(\cos^2\theta - 1)/2$. The same angular dependence was observed in spectra of perdeuterated hexane dissolved in DMPC bilayers (see Figure 11). The two extreme angles are shown in Figures 10 and 11 where, for all peaks observed, $\Delta\nu(0°) = 2\Delta\nu(90°)$. The truncated, slightly off center peak in Figure 11 arises from HOD.

Discussion

The presence of an isotropic line in the acyl-chain perdeuterated DMPC ^{2}H NMR spectra at high hexane concentrations (see Figure 9) indicates that some of the lipid is not in a bilayer arrangement. This is to be expected, since, in the limit where the lipid to hexane ratio approaches zero, the mixture is simply lipid dissolved in hexane with extraneous water. Therefore, the following discussion will be limited to those hexane concentrations in which the bilayer structure is intact.

We take the absence of change in the ^{31}P NMR spectra upon addition of up to 26 mol % hexane as evidence that the bilayer structure per se is not significantly perturbed by the presence of this much hexane. This precludes explaining the heat absorption in Figure 1 as lamellar–hexagonal or lamellar–micellar transitions.

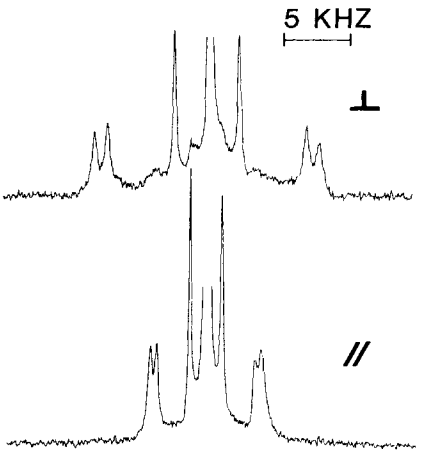

Figure 11. ^{2}H NMR spectra of 26 mol % perdeuterated hexane in macroscopically oriented multilayers of DMPC bilayers. The bilayer planes are oriented normal to the static magnetic field in the upper trace and parallel to it in the lower trace.

The most significant difference in the ^{2}H NMR spectra of per-^{2}H-DMPC with and without hexane is that the system with hexane maintains a sharp liquid crystalline type line shape to lower temperatures. As Figure 5 shows, the addition of hexane causes only a small decrease in the lipid chain order. A similar lack of perturbation of the bilayer by benzyl alcohol has been observed.[24] Likewise, neutron and X-ray diffraction studies have shown that little bilayer perturbation is caused by halothane, nitrous oxide, or cyclopropane.[25]

It has been observed that gaseous anesthetics tend to lower and broaden the lipid bilayer gel-to-liquid crystalline phase transition.[26-28] Jain and co-workers[26] noted the same behavior for a variety of local anesthetics. A large low-temperature heat absorption event like that seen in Figure 1 was not observed with the anesthetics. The different thermal characteristics may be a concentration effect rather than a manifestation of qualitatively different lipid behavior. Unfortunately, the actual concentration of anesthetic in the bilayer was not determined in these studies. One must also consider that the local anesthetics have an amphipathic character that could cause the phase behavior to be different.

The thermograms observed in the DMPC/hexane mixtures resemble the results obtained by Mabrey and Sturtevant[13] for lipid/lipid bilayer mixtures. Since the NMR spectra of the lipid molecules indicates that the bilayer structure is conserved at all temperatures examined and at hexane concentrations up to 26 mol %, it is valid to discuss the phase behavior of the *two-component* bilayer mixture (within the temperature and concentration limits noted) just as the phase behavior of *one-component* bilayers is discussed, even though in both cases water is an additional component without which no bilayer would form.[13,29] The excess water is a separate phase and does not enter into this discussion.[30] In this case the low-temperature "onset" of heat absorption separates the low-temperature region labeled C in Figure 2 from the intermediate-temperature region B, while the temperature at which the high-temperature peak reaches "completion" describes the dividing line between region B and the high-temperature region A. Figure 2 thus represents a partial phase diagram for the hexane/DMPC bilayer mixture. It most closely resembles the DSPC/DLPC bilayer mixture (acyl chain length difference of six carbons) in which monotectic behavior is observed. In such

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a phase diagram region A corresponds to a single homogeneous phase (liquid crystalline bilayer). Region B contains two phases (one hexane rich and liquid crystalline-like, the other hexane poor and gel-like). If the onset temperatures describe a horizontal line, region C also contains two phases (both gel-like, one hexane rich and the other hexane poor) *and* there must be a single phase region D in the low hexane content portion of the phase diagram. The curve separating regions D and A will intersect the ordinate at the melting temperature of the pure lipid bilayer, touch the onset temperature line at its end point, and continue to fall, thus separating regions D and C.[31] The dashed line in Figure 2 is drawn to approximate these condtions, although it is not known at what mol % hexane the onset temperature line terminates. If the onset temperatures do not actually describe a horizontal line, then regions D and C merge into one region containing a single homogeneous gel-like phase.

The hexane ^2H NMR data support the above interpretation of the DSC thermograms and provide information about the behavior of the alkane in the various phases. The disappearance of the powder patterns and concomitant appearance of the central isotropic line at approximately the isothermal melt temperature support the above conclusion concerning the existence of a low-temperature hexane-rich phase. The lack of any observable heat absorption in the DSC thermograms at or near the freezing point of hexane (−95 °C) suggests that this hexane-rich phase is not pure hexane, even though the hexane motion is isotropic. The interface between regions A and B in the phase diagram is apparent in the ^2H NMR data as the maxima in plots of $\Delta\nu_q$ vs. temperature for the different hexane concentrations. The powder patterns also exhibit an apparent broadening as the temperature is lowered through region B. This broadening is not accompanied by a shortening of T_{2echo} of the innermost powder pattern peaks (see Table II). A broadening due to a distribution of $\Delta\nu_q$'s is consistent with these observations.

The hexane ^2H NMR powder patterns give direct information about the average order imposed upon the alkane by the bilayer and, by inference, information about the types of anisotropic motion being executed. A comparison of the hexane and lipid acyl-chain quadrupole splittings above 27 °C reveals that the hexane methyl and methylene units (maximum $\Delta\nu_q$ = 10 kHz) are significantly less ordered than any of the DMPC methylene units (minimum $\Delta\nu_q$ = 20 KHz). Only the lipid methyl group with its relatively unrestricted rotation ($\Delta\nu_q$ = 4 kHz) is as disordered as any part of the hexane molecule. That only three powder patterns are observed from per-^2H-hexane is somewhat surprising. If the hexane molecules align themselves parallel to the lipid chains, and if the order parameter gradient found in lipid bilayers[23,32] is imposed on hexane, then one would expect the ^2H NMR spectrum of per-^2H-hexane in this environment to contain six overlapping powder patterns and the methyl-deuterated hexane spectrum to contain two powder patterns. That a single powder pattern is observed in the methyl-deuterated case and only three powder patterns for per-^2H-hexane implies that both ends of the molecule are experiencing the same average environment. That the orientation dependence of both the lipid and hexane ^2H NMR spectra in macroscopically aligned bilayer samples is the same implies that the direction of motional averaging for the hexane molecules is normal to the plane of the bilayer. Why both ends of the hexane molecule experience the same average environment must remain, for the present, a matter of conjecture. Two possibilities are (1) shuffling of hexane parallel to the acyl chains between the monolayers and (2) rapid rotation of the alkane about an axis normal to the plane of the bilayer. In either case the motion must be fast on the NMR timescale.

Conclusions

Taken as a whole, the DSC and NMR results yield a relatively clear picture of the DMPC/hexane/(excess water) system. The lipid deuterium and phosphorus NMR spectra are remarkably insensitive to the addition of large amounts of hexane to the bilayer, while the DSC thermograms of the mixtures are quite sensitive to the amount of the alkane in the bilayer. Moreover, the hexane ^2H NMR spectral characteristics are strong functions of both temperature and hexane content. We draw the following conclusions. (1) Upon the addition of up to one hexane molecule per two lipid molecules the lipids in the bilayer are not significantly perturbed by the presence of the alkane. (2) The hexane is disordered relative to the surrounding bilayer and both ends of the alkane experience the same average environment. (3) The mixture exhibits isothermal melting at approximately 0 °C, indicating the existence of a low-temperature phase separation in which hexane-rich and hexane-poor phases form. (4) In the hexane-rich low-temperature phase the motion of the hexane molecules is isotropic.

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Registry No. DMPC, 13699-48-4; hexane, 110-54-3; [1-$^3H_1$]hexane, 3675-57-8; [1-$^2H_1$]hexane, 4206-30-8; 1-bromohexane, 111-25-1.

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