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Alcohol interactions with lipids: a carbon-13 nuclear magnetic resonance study using butanol labeled at C-1

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The interactions of carbon-13 enriched butanol with dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) were studied using C-13 nuclear magnetic resonance. It was found that above the gel to liquid crystal phase transition the resonance from the butanol could be resolved into two signals with similar chemical shifts but different T_1 values and line widths. In contrast, only one narrow resonance was observed for ethanol, which has considerably less solubility in the lipids than butanol. Thermodynamic analyses of the effects of butanol on the phase transition temperature predict much greater solubility for butanol when the lipid is above the phase transition temperature than when it is below. It was concluded that the two butanol resonances represent two slowly exchanging populations, the free butanol in the aqueous phase and butanol dissolved in the liquid crystalline region of the lipid. No bound butanol was detected below the gel to liquid crystal phase transition. Relaxation studies were performed on the resonance of the bound butanol in DPPC and DMPC, including measurements of T_1, line width, and nuclear Overhauser enhancement. Theoretical analysis of the relaxation parameters indicates that the lipid-bound alcohol has very high mobility within the fluid lipid bilayer. The data are consistent with butanol being present at the aqueous boundary or head group region of the lipid.

Introduction

The role of lipid physical properties in the function of biological membranes has not been adequately established. Although the lipid specificity of membranes has long been observed, the molecular basis for a functional explanation of this is elusive. General anesthetics, including alcohols, dissolve in the lipids of membranes and apparently alter the function by altering the lipid properties [1,2]. Thus, studies of the effects of small perturbants and anesthetics on the physical properties of lipids can provide insight, not only into the mechanisms of action of these compounds, but also into the role of lipid properties in membranes.

We have been investigating the physical interactions of alcohols with synthetic lipids in order to

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Abbreviations: PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; NOE, nuclear Overhauser effect.

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characterize the effects on the phase equilibria and lateral phase separations of lipids [3–7]. We have found that alcohols lower the gel to liquid crystal phase transitions of PC and PE at low alcohol concentrations and that these shifts can be interpreted in terms of ideal solution theory, yielding the membrane-buffer partition coefficients for the alcohol in the liquid crystal phase [3,4]. The thermodynamic results of the analysis of the T_m shift indicate that the alcohol is more soluble in the liquid crystal phase than in the gel phase.

Nuclear magnetic resonance has been used in several instances to investigate the interactions of anesthetics with lipids using various nuclei [7–13]. We have previously used ^{13}C-NMR to investigate the interactions of relatively high concentrations of butanol with DPPC in the gel phase [7]. In the present investigation ^{13}C-NMR has been used to directly study ^{13}C-enriched ethanol and butanol in the presence of DPPC and DMPC at temperatures above and below the phase transition temperature. Relatively low alcohol concentrations were examined where ideal solution theory is applicable. The purpose of our study was to focus on the dynamics and the properties of the alcohols as they interact with the lipid. Based on known partition coefficients, conditions were chosen where no significant alcohol lipid solubility was expected for both ethanol and butanol (i.e. below the phase transition temperature), and where only butanol was expected to be significantly soluble in the liquid crystal phase (i.e. above the lipid phase transition). These investigations have resulted in the identification of a resonance arising from the lipid-dissolved butanol. This resonance was studied in terms of the motional properties of the lipid-dissolved butanol.

Methods

Lipids. DPPC and DMPC were obtained from Calbiochem or Sigma. They were used without further purification after checking the purity by thin-layer chromatography. Aqueous multilamellar liposome suspensions were prepared according to the method of Bangham et al. [14]. Lipid concentrations for the NMR experiments were approx. 200 mg/ml in a solvent of 0.001 M EDTA and 10% ^2H_2O. The ^2H_2O was added to provide a deuterium signal for locking the NMR spectrometer and to help prevent settling of the lipid by adjusting the solvent density. For the spectrophotometric experiments the lipid concentration was approx. 0.55 mg/ml, and sucrose was added to improve the optical properties and reduce settling of the suspensions [15].

Labeled compounds. Ethanol and butanol selectively enriched to 90% in C-13 were obtained from KOR Isotopes, Inc. The ethanol was enriched at the C-2 (methyl) or the C-1 position, and the butanol was enriched at the C-1 position.

Spectrophotometry. The effect of butanol on the gel to liquid crystal phase transition of DPPC was determined by following the phase transitions of dilute lipid suspensions by absorbance using a Varian/Cary 219 spectrophotometer, as described previously [3]. The Varian/Cary 219 was interfaced to an Apple IIe, and the temperature and absorbance data were collected simultaneously and stored on disk for further analysis and plotting. The temperature was controlled by circulating water from a programmable bath through jacketed cuvettes. The temperature probe was located in a reference cuvette connected in series with the sample cuvettes. Methodology for these measurements was as described previously [3].

Nuclear magnetic resonance. The NMR measurements were performed on a Bruker WP-200 spectrometer equipped with a 10 mm C-13 probe (50.32 MHz) and a Bruker BVT-1000 variable temperature unit capable of temperature control with a resolution of 1 Cdeg. The T_1 measurements were performed using the standard inversion recovery method, and the data were analyzed by a 3-parameter non-linear least-squares method. Typical waiting times used were about 60 s, and 8K data were collected. In the T_1 measurements on butanol in the presence of lipid above the phase transition, a subtraction procedure described below in Results (Figs. 5 and 6) was used for each tau value in order to resolve the overlapping signals from the bound and free alcohol. The 8K data were expanded to 16K using zero filling when needed to improve the accuracy of the subtraction. The nuclear Overhauser enhancement (NOE) was determined by using gated decoupling.

Results

Membrane-buffer partition coefficients

The membrane-buffer partition coefficient for butanol in DPPC was estimated by the T_m depression method [3]. As discussed previously, the value obtained by this method represents the difference in partition coefficient between the gel phase and the liquid crystal phase. It does not include any butanol which is bound equally to the two phases. If there is significant solubility of butanol in the gel phase then the partition coefficient determined by this method would be lower than that determined by more direct methods.

Table I shows the partition coefficients calculated by this method for butanol in DPPC along with various values from the literature determined by more direct methods. As seen here our value is in good agreement with the literature values. It should be noted that our calculations are based on the T_m depression of DPPC over the low butanol concentration range over which the T_m depression is linear (up to 8 mg/ml). At higher alcohol concentrations non-linear effects occur as the lipid goes into the interdigitated state [7]. These effects apparently involve an increase in butanol binding to the gel phase lipid, probably in the head group region.

Using the partition coefficients of Table I, one can calculate that at the lipid concentrations used in our NMR studies, between 25% and 75% of the butanol will be dissolved in the fluid lipid above the phase transition temperature. Also shown in Table I is the partition coefficient for ethanol into DPPC determined by the T_m depression method. From this partition coefficient it can be estimated that at the lipid concentration used in the NMR experiments, less than 5% of the ethanol will be dissolved in the lipid even above the phase transition temperature. Below the phase transition temperature no lipid solubility is expected for either of these alcohols.

TABLE I

MEMBRANE-BUFFER PARTITION COEFFICIENTS

Alcohol	Lipid	K_p [a]	K_w [b]	References
Butanol	DPPC	63.1	1.5	present study (T_m depression)
	DPPC	270–800	6–20	present study (NMR)
	DMPC	186–600	5–20	present study (NMR)
	DPPC	117		Hill [16]
	DMPC		3.16	Katz and Diamond [18]
	erythrocyte membranes		1.5	Roth and Seeman [17]
Ethanol	DPPC	4.3	0.1	Rowe [3]
	erythrocyte membranes		0.14	Roth and Seeman [17]

[a] K_p is the dimensionless mole fraction partition coefficients.
[b] K_w is in weight concentrations: mol alcohol/kg lipid : mol alcohol/liter solvent.

^{13}C-NMR studies

We used ^{13}C-enriched alcohols to determine whether the interactions of the alcohols with the lipid would result in a significant effect on the ^{13}C-NMR signal that could then be used to characterize the interactions. We chose butanol because it has a large enough partition coefficient in the liquid crystal phase to be significantly bound under the conditions of the NMR experiment. In order to demonstrate that the effects on the butanol resonances observed are indeed due to the interactions of butanol with the lipid, we have also examined the butanol resonance under conditions where little binding is expected, i.e., below the phase transition temperature. To further verify that the observed effects on the butanol resonance are due to interactions with the lipid, and not, for example, the effects of raised temperature, we have also included ^{13}C-enriched ethanol in some of the samples. As remarked above, because of its low partition coefficient, less than 5% of the total ethanol is bound even above the phase transition temperature. Thus, we are looking for an effect on the ^{13}C-butanol resonance which is specific for the condition above the lipid melting temperature and which does not occur with the ethanol signal.

Fig. 1 shows the ^{13}C-NMR spectrum of DPPC containing both 0.05 M butanol (^{13}C-enriched to 90% at the C-1 position) and 0.1 M ethanol (^{13}C-enriched to 90% at the C-2 position) at a temperature above the phase transition. It is seen that at the relative concentrations of lipid (200 mg/ml) and alcohol, the signals from the ^{13}C-enriched alcohols dominate the spectra. At this scale, the lipid choline methyl resonance is barely visible upfield of the butanol C-1 resonance. This demonstrates that the signals from the alcohols can be

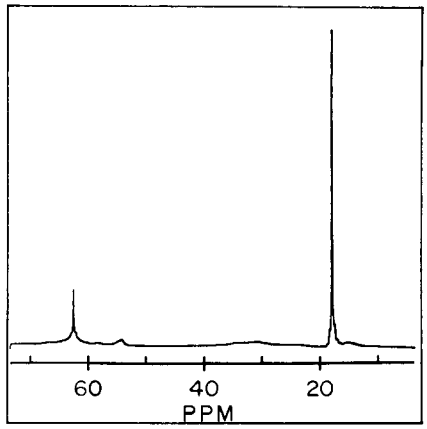

Fig. 1. The 50.32 MHz ^{13}C-NMR spectrum of 0.10 M ethanol enriched at the C-2 position (chemical shift of 17.8 ppm) and 0.05 M butanol enriched at the C-1 position (chemical shift of 62.5 ppm) in the presence of 200 mg/ml DPPC at 46 ° C.

readily investigated with relatively little interference from the natural abundance resonances of the lipid. In the quantitative studies of the alcohol signals, however, the lipid resonances were nevertheless completely eliminated by subtracting the spectrum of a lipid sample containing no alcohol.

Fig. 2 shows the ^{13}C resonances of the enriched butanol and ethanol above (42 ° C) and below (38 ° C) the DPPC phase transition. In the upper panel it is seen that the butanol resonance changes from a narrow symmetrical signal to one which has considerably less intensity and is broadened at the base over this 4 ° C temperature change. In contrast, the lower panel shows that there is relatively little change in the ethanol C-2 resonance during the lipid phase transition. Control studies showed that the ethanol C-1 resonance also shows little change. Fig. 3 shows a more detailed examination of the change in the butanol resonance, in which spectra were taken over a broad temperature range. As seen here, the butanol resonance changes abruptly at the T_m of the lipid and remains fairly constant for a wide range of temperatures on either side of the transition. The change in the butanol resonance which occurs at the lipid phase transition is reversible. As seen in Fig. 3, reducing the temperature restores the original intensity and the shape of the signal. The C-2 resonance of the ethanol was also followed and found to exhibit little change over the temperature range (data not shown). These results suggest that the

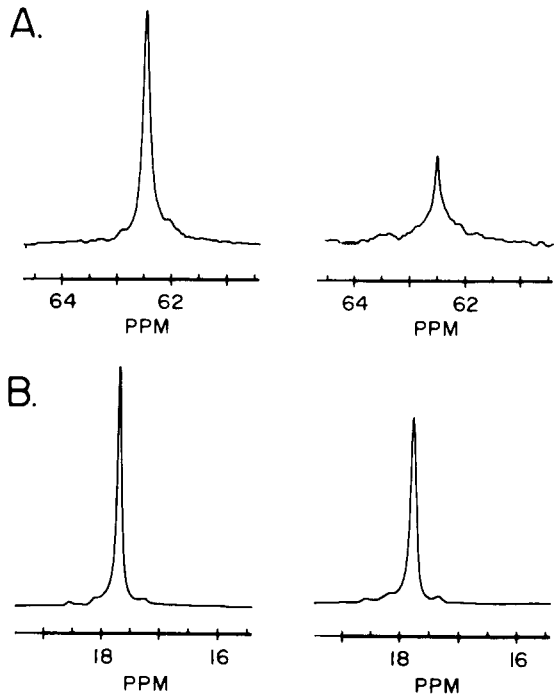

Fig. 2. The effect of the DPPC phase transition on the alcohol resonances. (A) The C-1 butanol resonance at 38 ° C (left) and at 42 ° C (right). (B) The C-2 ethanol resonance at 38 ° C (left) and at 42 ° C (right).

change in the butanol signal at the lipid phase transition is due to the solubility of the butanol in the lipid above the phase transition. In each of the spectra shown in Figs. 2 and 3 the natural abundance spectrum of the lipid has been eliminated by subtraction.

To further characterize the nature of the change in the butanol resonance as a function of the phase state of the lipid, the integral of the butanol resonance is plotted in Fig. 4 as a function of temperature (lower panel). The integral of the ethanol signal from the same sample is shown in the upper panel. Both were normalized to give the same area at 30 ° C to facilitate comparison. As seen here there is no discontinuous change in the integral of the resonance from either alcohol at the lipid phase transition temperature. For the butanol data, the reversibility of the measurements is indicated by the closed symbols. For both alcohols there is a linear decrease in total integral with increasing temperature, and the negative slope is greater for the butanol than for the ethanol. The

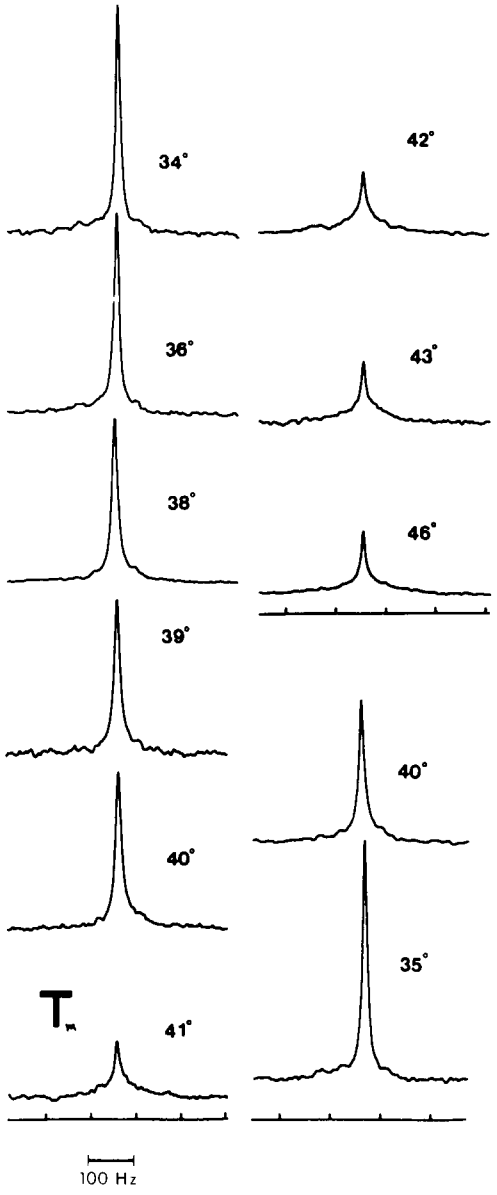

Fig. 3. The C-1 butanol resonance as a function of temperature in the presence of DPPC, measured in order of ascending temperature through the phase transition. The two bottom right hand spectra were taken after temperature reversal.

origin of this negative slope has not been elucidated, and it must be borne in mind that it could reflect a temperature-dependent population of highly immobilized butanol that is not observable by ^{13}C-NMR at present. Another possibility is settling or floating of the lipid that varies with temperature due to density changes in solvent or

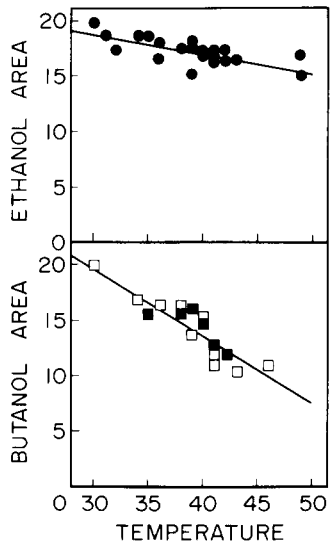

Fig. 4. Total integrals for the C-1 butanol and C-2 ethanol resonances in the presence of DPPC as a function of temperature. The integrals were normalized to the same area at 30 °C. The closed symbols for butanol represent reversibility experiments in which the measurement was made after heating the sample to 46 °C.

lipid. The fact that there is no discontinuous change in the butanol integral at the phase transition shows that the intensity change seen in Figs. 2 and 3 is due entirely to a change in shape of the butanol resonance as the butanol dissolves into the fluid lipid.

The shape of the butanol resonance above the lipid phase transition was closely examined. Fig. 5A shows an expansion of a typical butanol resonance above the DPPC phase transition (46 °C). For each of these spectra the natural abundance resonances of the lipid were eliminated by subtraction of the natural abundance spectrum of a lipid control. It is evident in this enlarged view that although it appears to be a sharp peak, it is considerably broadened at the base, suggesting a superposition of a narrow signal with a broad one. This broad underlying signal does not come from contributions due to the natural abundance signals of the lipid, since it is present after subtraction of the spectrum of lipid alone. Resolution of this butanol C-1 resonance into two overlapping resonances having the same chemical shift but very different line widths has been carried out. Fig. 5C shows the resonance of butanol in the

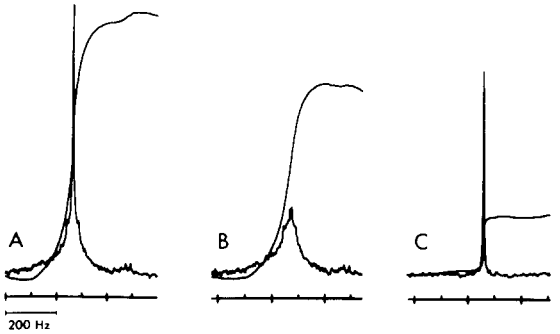

Fig. 5. Resolution of the [^{13}C]butanol signal in the presence of lipid into two signals above the DPPC phase transition at 46 ° C after subtraction of the lipid spectrum. (A) Total butanol signal. (B) Difference between A and C. (C) The signal of butanol in the absence of lipid, scaled to optimize the removal of the narrow component in A.

absence of lipid, scaled so that the subtraction of this signal from that of Fig. 5A optimizes the removal of the narrow component in the latter. Fig. 5B shows the remaining broad signal after the subtraction. The line width of the broad component was approx. 70 Hz, while the line width of the sharp component is approx. 5 Hz. The integrals, which are also shown in Fig. 5, indicate that the broad signal accounts for approximately two-thirds of the total butanol signal. Similar results were obtained for butanol in DMPC at 32 ° C, just above its transition temperature. The ability to resolve these resonances into two overlapping resonances most simply suggests that the butanol is present in two distinct environments and that exchange between these two environments is not fast on the NMR time scale.

A similar subtraction process applied to the butanol signal in DPPC at 30 ° C, below its phase transition, leads to very different results as shown in Fig. 6. At least 80–90% of the total butanol signal is accounted for by the free butanol signal. The difference remaining after subtraction is not above the experimental error of matching the free butanol component for the subtraction process, so this data does not support the presence of any broad underlying peak. A similar subtraction process was applied to the ethanol resonance in the same samples, and no significant underlying peak was found for the ethanol signal at any temperature. This comparison is significant in showing that the butanol effect is a real result of a change

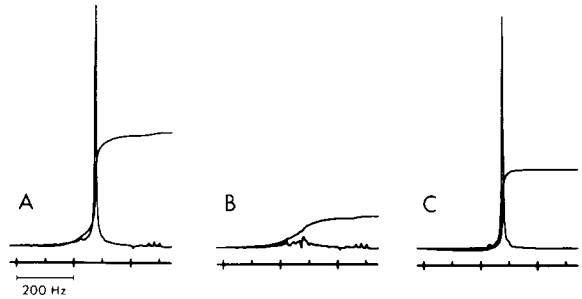

Fig. 6. Resolution of [^{13}C]butanol in the presence of DPPC into two signals at 32 ° C below the lipid phase transition. A–C are as described in the legend to Fig. 5.

in its interaction with the lipid at the lipid phase transition and not a generalized effect on the solution.

Fig. 7 shows plots of the integrals of the two resolved butanol resonances as a function of temperature along with the total butanol integrals (from Fig. 4). In contrast to the monotonic temperature effect on the total butanol integral it is seen that there is a discontinuous change in the proportion of the two signals at the lipid transition temperature. The integral of the broad signal stays constant up to 41 ° C, and then abruptly increases over a one degree range to a fairly

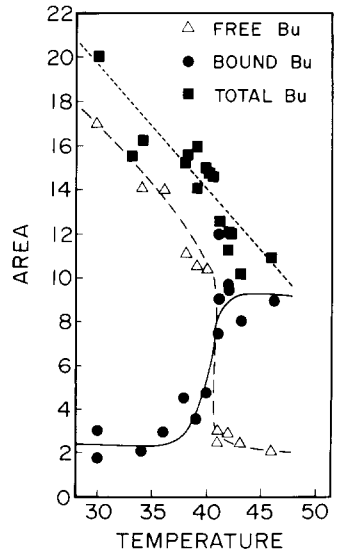

Fig. 7. Integrals of the resolved broad ('bound') and sharp ('free') components of the butanol resonance as a function of temperature.

constant higher level. At the same time the integral of the sharp portion of the signal decreases correspondingly. This pattern is consistent with the interpretation that the two populations represented by the two resolved signals are butanol which is present dissolved in the lipid and that in the aqueous phase. The sharp change in the relative proportions of these two populations is consistent with the expectations based on the partition coefficients.

The quantitation of the proportions of the butanol present in the two populations by these integrals requires the measurement of the nuclear Overhauser enhancement (NOE) for butanol in the free and bound states. The NOE was thus obtained for the ethanol and butanol signals above and below the transition. In the presence of DPPC, the C-1 of butanol exhibited an NOE of 3.2 at 29°C, while a value of 3.4 was obtained at 51°C where the broad resonance dominates the integral. For ethanol in the presence of DPPC, the NOE was 2.7 at both 29°C and 51°C. It is thus seen that even though the butanol signal is broadened, the NOE is still within experimental error of the theoretical dipolar maximum of 2.98 under all conditions. Thus no corrections to the areas are needed in order to relate area to concentration.

We have used the relative areas of the resolved signals to estimate NMR membrane-buffer partition coefficients for butanol in DMPC and DPPC above the phase transition, and this is shown in Table I along with the other values. The large uncertainties in the subtraction process and in the integration of the signals lead to the range of values shown in Table I. The values obtained are larger than, though of the same order of magnitude as, the published values and the value obtained by the transition temperature depression calculations. One possible explanation for the higher NMR value is that the lipid suspension is not homogeneously distributed in the NMR tube; considerable lipid settling or floating sometimes occurs. Thus the lipid concentration inside the receiver coil can be greater than the overall lipid concentration, giving rise to a greater proportion of the area from the bound alcohol. The results obtained for DMPC and DPPC were similar. In order to further establish that the observed effect was due to the partitioning of butanol into the lipid, the concentrations of butanol and DMPC were varied. The lipid was varied over a 2-fold range, and the butanol was varied over a 5-fold range; the calculated partition coefficient remained within the range of 12–20.

^{13}C-NMR relaxation studies

T_1 relaxation studies were done on the butanol signal in DPPC above and below the main transition temperature and in DMPC above the transition temperature. A typical example of a T_1 inversion recovery experiment on butanol in DMPC is shown in Fig. 8. It is very important to note the two-component nature of the butanol signal revealed in the partially relaxed spectrum for tau of 3 seconds. The underlying broad component of the signal has passed the null point and become positive while the narrow component is still inverted.

The T_1 relaxation times were measured for the two butanol components, using at each tau the subtraction procedure illustrated in Fig. 5. The results of these measurements are given in Table II. As seen there, the broad ('bound') component in DPPC at 51°C has a T_1 of 2.0 s. In DMPC at 32°C the broad component has a T_1 of 1.5 s. The narrow ('free') component has a T_1 of 8.5 s at

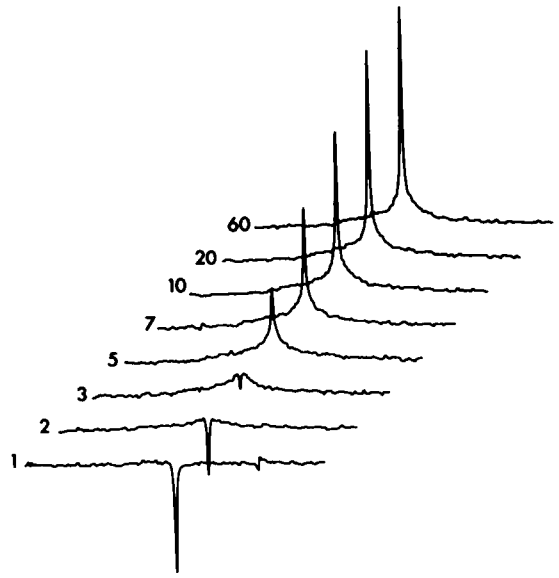

Fig. 8. Inversion-recovery spectra for enriched butanol in DMPC at 32°C. The delay between the 180° and 90° pulses is indicated in the figure in seconds.

TABLE II

BUTANOL C-1 SPIN-LATTICE RELAXATION TIMES

Sample	Temperature (°C)	T_1 (s)
Butanol alone	35	6.9±0.1
	51	10.0±0.2
Total butanol signal		
In DPPC	29	5.2±0.2
	35	5.0±0.2
	46	6.9±0.2
	51	8.5±0.2
In DMPC	32	4.7±0.2
Resolved resonances		
Broad component in DPPC	51	2.0±0.5
Broad component in DMPC	32	1.5±0.4
	32	1.7±0.4
Narrow component in DPPC	51	8.5±0.1

51°C as compared to 10.0 s for butanol alone. The observation of distinct relaxation times for the broad and narrow resonances provides excellent evidence for the assumption that they arise from two environments that are in more or less slow exchange. Furthermore, the rate of interconversion between the environments must be less than the reciprocal of these T_1 relaxation times * (cf. Ref. 26).

Discussion

The present investigation has successfully detected two distinct NMR resonances of ^{13}C-enriched butanol in the presence of liquid crystalline lipid. Several lines of evidence indicate that these resonances reflect butanol in the free aqueous phase and butanol that is dissolved in the liquid crystalline lipid. The broad resonance thus appears at the expense of the narrow resonance during the lipid phase transition, as shown by the approximate constancy of the total integral during the narrow transition. The broad component appears only in the presence of lipids that are above the gel to liquid crystal phase transition temperature (24°C for DMPC and 41°C for DPPC). This occurs reversibly, since cooling the lipid to below the phase transition leads to the disappearance of the resonance. This is consistent with the thermodynamic analysis of the effect of butanol on the phase transition temperature, since such analysis predicts appreciably greater butanol solubility in the liquid crystalline state. Indeed, the membrane-buffer partition coefficient calculated from the NMR experiment is generally similar though somewhat greater than that independently determined by other methods such as melting temperature depression or from direct binding studies [16–18]. The absence of a broad resonance when ^{13}C-labeled ethanol is examined provides excellent confirmation of these conclusions, since examination of its known membrane-buffer partition coefficient predicts solubility in the liquid crystalline phase which is below the limits of detection by our method.

The observation of distinct resonances from distinct environments has been previously reported for other anesthetics, such as in ^{19}F-NMR studies of halothane interactions with membranes [10], or the binding of tetracaine to egg phosphatidylethanolamine that was studied by ^{2}H-NMR [8]. This situation provides us with an opportunity to characterize the various environments and interactions of butanol within the liquid crystalline lipid. The subtraction procedure described above has permitted us to independently examine the narrow and broad butanol C-1 resonances. The spectra (Fig. 5) reveal that there is no significant chemical shift difference between butanol in the two environments. It is reasonable to infer from this that the butanol C-1 in the liquid crystalline environment is in an environment that is not too different from the aqueous one, i.e. that lipid-dissolved butanol is oriented with its hydroxyl group exposed to the solvent or to the phospholipid head groups. However, the chemical shift data alone cannot prove this.

More direct information on the environment of the butanol can be obtained from the dynamic NMR measurements we have carried out. Inver-

* To the extent that the broad and narrow butanol resonances are not in the limit of slow exchange, the observed T_1 values will be lower limits of the true T_1 values (cf. Ref. 26). This follows because the kinetic rate of exchange between the environments (k_{exch}) will contribute to the observed T_1 according to: $(1/T_1)_{obs} = (1/T_1) + k_{exch}$. The k_{exch} correction cannot be dominant, otherwise the T_1 values for the narrow and broad components would have been identical.

sion-recovery studies reveal distinct spin-lattice relaxation rates for the narrow (free) and broad (lipid-dissolved) resonances above the phase transition temperature (Table II). These data indicate that the two butanol populations are in approximately slow exchange on the NMR time scale. They thus support the attribution to different environments and rule out more complex explanations for the unusual overall line shape [19,20]. The dynamics of the lipid-dissolved butanol can be examined within a dipolar model for the relaxation. The greater line width and shorter T_1 of the bound butanol signal relative to the free signal suggests that the lipid-dissolved butanol is less mobile than free butanol, but the large NOE suggests that the bound butanol still has considerable mobility.

We have carried out a more quantitative analysis of these observed relaxation parameters (T_1, T_2, and NOE). We have chosen a simple diffusional model of dipolar relaxation in which a free internal rotation is assumed to occur along a molecular axis of butanol in addition to an overall isotropic re-orientation of the liposomes. The C-1 carbon nucleus is assumed to be relaxed by the two covalently attached protons. Such models have been shown to yield T_1 and NOE values that are insensitive to the overall re-orientation of the particles (characterized by a correlation time τ_R) when such re-orientation is much slower than the internal rotation (characterized by a correlation time τ_G) [21]. Other relaxational models may be invoked (cf. Refs. 22, 23) but the present simplistic one provides a readily interpretable description of the general features of the dynamics. Similar models have been used in the past to discuss motion of membrane-bound molecules [24]. Equations for the present model have been previously derived (cf. Refs. 21, 25) and are not repeated here. We note, however, that the equations are similar to those of the rigid isotropic rotation case, except that the so-called spectral density functions become functions of the internal motion correlation time (τ_G) and the angle θ between the C-H dipolar vector and the axis of internal rotation.

Numerical calculations using the above model in which τ_G and θ are both varied quickly reveal that the observed large NOE value is only compatible with very rapid internal rotational correlation

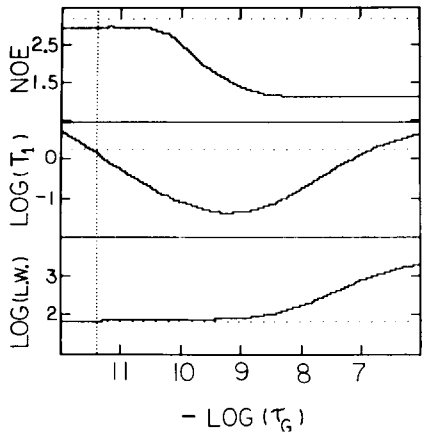

Fig. 9. Dependence of the dipolar ^{13}C-relaxation parameters NOE, T_1 (s) and line width (Hz) (bottom) on the correlation time τ_G for internal rotation. In each case, the experimentally observed values are indicated by the dashed horizontal lines. The theoretical curves are dipolar calculations (see Text) that utilized a magnetic field of 4.7 tesla, two bonded hydrogens at a distance of 1.084 Å, an overall isotropic re-orientation correlation time τ_R of $1 \cdot 10^{-6}$ s^{-1}·rad^{-1}, and an angle θ between the C-H bonds and the axis of internal rotation of 61.5° (or equivalently 118.5°). The vertical dashed line indicates a τ_G value that leads to a satisfactory fit to all three observed parameters.

times (τ_G) of the order of $1 \cdot 10^{-10}$ s$^{-1} \cdot$ rad^{-1} or shorter. The observed T_1 can be reproduced * using τ_G values in the range of $(2\text{–}3) \cdot 10^{-12}$ s$^{-1} \cdot$ rad^{-1}. This is shown by the calculations of Fig. 9, where τ_R was taken as $1 \cdot 10^{-6}$ s$^{-1} \cdot$ rad^{-1}, appropriate for sonicated vesicles [23,24] and a θ angle of 118.5° (see below). The computed NOE and T_1 values are not too dependent on the choice of the overall reorientation time τ_R or θ, as expected [21,25].

In contrast to the case of the NOE and the T_1, the calculations show that the computed line width is extremely sensitive to the choice of the angle θ between the dipolar vector (i.e. the C-H bonds at C-1) and the (unknown) axis of internal rotation. Fig. 10 shows this angular dependence whose consequence is that only an extremely narrow range of choices θ reproduce the observable line width of about 70 Hz that is shown by the horizontal dashed line. For example, in the (equivalent) θ ranges of 20–70° and 100–160°, each roughly

* See footnote on p. 158.

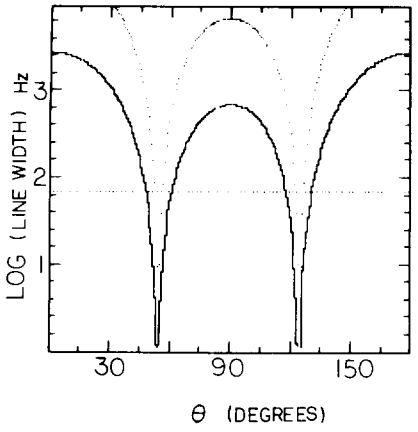

Fig. 10. Dependence of the logarithm of the computed dipolar line width for a carbon directly bonded to two hydrogens at a distance of 1.084 Å away on the angle θ between the C-H bonds and the axis of internal rotation. The theoretical calculation (see text) utilized a magnetic field of 4.7 tesla and an overall isotropic re-orientation correlation time τ_R of either $1 \cdot 10^{-6}$ $s^{-1} \cdot rad^{-1}$ (solid curve) or $1 \cdot 10^{-5}$ $s^{-1} \cdot rad^{-1}$ (dotted curve). The correlation time for internal rotation τ_G was fixed at $2.8 \cdot 10^{-12}$ $s^{-1} \cdot rad^{-1}$ in both cases. The horizontal dotted line indicates the experimental line width found for butanol in the presence of DPPC above the phase transition temperature, so that its intersection with the curves represents acceptable θ values that reproduce the observations.

centered around the dipolar 'magic angles' (54.74° and 126.26°) (Fig. 10), the line width varies over several orders of magnitude, while the computed T_1 varies only between 1.5 and 3.0 s and the NOE varies only between 2.4 and 3.0. It is interesting that a similar sensitivity to orientation has been recognized in studies of cholesterol–lipid interactions in which a more extended theoretical model was utilized [23].

The above results have interesting implications to understanding the dynamics of butanol when dissolved in the membrane above the phase transition. It clearly has extremely rapid internal motion, with correlation times shorter than $1 \cdot 10^{-11}$ $s^{-1} \cdot rad^{-1}$. Considerable fluidity is thus indicated, but this is not unreasonable, since it is an order of magnitude faster than found for the larger molecule cholesterol by ^{13}C-NMR [23,24]. Despite recognized limitations of the theoretical model used and the uncertainty in the choice of parameters for it, it is clear that butanol cannot be in a fully extended conformation while undergoing axial rotation along the long molecular axis. Fig.

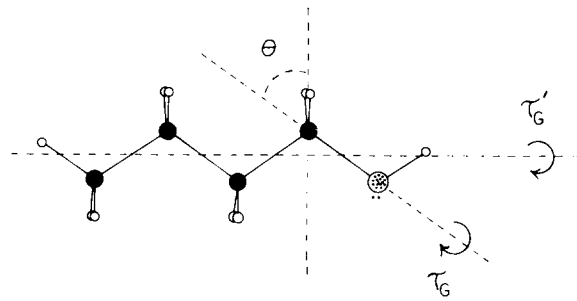

Fig. 11. Possible internal rotations affecting the C-H dipolar vector at C-1 of butanol. The axis of internal rotation of an extended butanol conformation is indicated by the horizontal dashed line (labeled by τ'_G). Rotation along the C_1-O bond is labeled by τ_G. This latter rotation would make an angle θ of 109.47° (tetrahedral angle) with the C_1-H vectors, while the former rotation would make a 90° angle.

11 shows that such a conformation and motion would produce a θ angle of 90° between the C-H vectors at C-1 and the long axis of the molecule. Fig. 10 shows that when τ_R of $1 \cdot 10^{-6}$ $s^{-1} \cdot rad^{-1}$ is assumed, acceptable angles that fit the observed line width are about 48.5° and 61.5° and their complements 131.5° and 118.5°.

It is important to note that the τ_R value utilized above should be treated as a lower limit, since the liposomes we used were unsonicated. The computed line width in the limit of fast internal rotation is actually linearly dependent on the value of τ_R, as is shown by comparing the dotted curve in Fig. 10 that was computed for a τ_R of $1 \cdot 10^{-5}$ $s^{-1} \cdot rad^{-1}$ with the solid curve. The acceptable angles stated above to fit the line width would thus converge closer to the 'magic angles' if larger τ_R values are applicable. Within the limitations of these uncertainties, our results may have significant implications. Our data could be consistent with the hydroxyl group being engaged in an interaction such as strong hydrogen bonding that anchors it, with resulting free rotation around the C_1-O bond. The θ value for such a situation (Fig. 11) would be the tetrahedral angle, i.e. 109.47°, which is within 15° of one of the acceptable solutions found to reproduce our line width observations.

Our present findings are complementary to and in excellent accord with a recent deuterium NMR study of the interaction of *n*-alkanes and *n*-alcohols with DMPC [9]. Using labeled butanol,

Pope and Dubro found a mobility gradient within butanol, but not in *n*-alkanes, in the presence of lipid, such that the motion increased with distance from the hydroxyl group. They thus suggested that butanol is anchored at the surface of the bilayer. Further ^{13}C-NMR studies on butanol labeled at the other carbons may help define the overall orientation and may quantitate individual diffusional parameters for rotation around all the bonds, thus complementing the order parameter determinations by ^{2}H-NMR.

The present results are also particularly interesting in comparison with those of our earlier study of the effects of butanol on the induction of the interdigitated gel phase of DPPC [7]. In that work, much higher concentrations of butanol were used, and the effects were examined below the gel to liquid crystalline transition. Although thermodynamic evidence was obtained that butanol interacts with the interdigitated gel phase, no change in the shape of the butanol C-1 resonance was found. It is possible that any butanol 'bound' to the interdigitated phase was in rapid exchange with bulk butanol, but this is rendered less likely by the observation that the T_1 of butanol was unaffected. On the other hand, it was observed at very high butanol concentrations that the total integral of the butanol resonance did not increase in direct proportion to the increase in butanol concentration. This raises the possibility that butanol in the interdigitated phase may be sufficiently tightly bound and broadened so as to escape NMR detection. Multiple environments and properties of membrane-dissolved anesthetics have been previously observed, such as in the ^{19}F-NMR studies on halothane [10].

In summary, we have successfully utilized ^{13}C-NMR to directly detect for the first time what may be lipid-bound butanol in both DPPC and DMPC. The ability to do so appears to be linked to the membrane-buffer partition coefficient of this alcohol, since no such binding was observed with ethanol. Analysis of the NMR relaxation properties of the enriched butanol indicate a considerable amount of motional freedom of the lipid-bound alcohol. Further studies are needed to define the orientation of the alcohol chain within the lipid and to determine its distance from the headgroups.

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