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LOW-FREQUENCY MOTION IN MEMBRANES

THE EFFECT OF CHOLESTEROL AND PROTEINS

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Nuclear magnetic resonance (NMR) relaxation techniques have been used to study the effect of lipid-protein interactions on the dynamics of membrane lipids. Proton enhanced (PE) ^{13}C-NMR measurements are reported for the methylene chain resonances in red blood cell membranes and their lipid extracts. For comparison similar measurements have been made of phospholipid dispersions containing cholesterol and the polypeptide gramicidin A^+. It is found that the spin-lattice relaxation time in the rotating reference frame ($T_{1\rho}$) is far more sensitive to protein, gramicidin A^+ or cholesterol content than is the laboratory frame relaxation time (T_1). Based on this data it is concluded that the addition of the second component to a lipid bilayer produces a low-frequency motion in the region of 10^5 to 10^7 Hz within the membrane lipid. The $T_{1\rho}$ for the superimposed resonance peaks derived from all parts of the phospholipid chain are all influenced in the same manner suggesting that the low frequency motion involves collective movements of large segments of the hydrocarbon chain. Because of the molecular co-operativity implied in this type of motion and the greater sensitivity of $T_{1\rho}$ to the effects of lipid-protein interactions generally, it is proposed that these low-frequency perturbations are felt at a greater distance from the protein than those at higher frequencies which dominate T_1.

Introduction

Many properties of membrane function are thought to depend upon the structure, organisation and fluidity of the membrane lipid. Cellular functions as varied as growth [1], active transport [2] and respiration [3] have all been shown to depend upon the physical state of the membrane lipids. The probable mechanism for this effect is through the interaction of the membrane lipid with the intrinsic membrane protein. A variety of techniques have now been employed to study the influence on the dynamics of the membrane lipid of many different proteins. In particular extensive use has been made of electron spin resonance (ESR) [4–6], fluorescence probe [7,8] and NMR linewidth and relaxation [9–11] techniques. The results obtained using these different approaches have so far proven to yield quite different information on the dynamic state of the lipids adjacent to the membrane protein. Models based on results derived from the earlier ESR data [4,5] have had to be substantially modified to incorporate more recent data obtained using NMR. However no single model appears to provide a general explanation of all of the effects seen to occur as a result of the lipid-protein interactions.

Oldfield et al. [12,13,14] have questioned the more traditional view developed from ESR and fluorescence data that surrounding each protein

molecule there is a boundary layer of lipid which is immobilised and distinct from the lipid elsewhere in the membrane. Based on ^{2}H-NMR measurements of specifically deuterated phospholipids [12,14] it was shown that cytochrome oxidase, cytochrome b_5, the total protein of *Escherichia coli*, bacteriophage f1 coat protein, the proteolipid protein of myelin and the polypeptide gramicidin A^+ all cause a disordering of the surrounding lipid. The extent of this disorder depended upon the protein studied and was greatest for gramicidin A^+. However, in all cases only a single averaged NMR doublet was observed, indicating that the lipid molecules were rapidly exchanging between the sites available to them on the timescale defined by the NMR experiment. Similar results have been reported using ^{31}P-NMR [15].

To be effective in averaging the NMR splitting the molecular exchange needs to be rapid in comparison with the observed NMR splitting. The difference between the ESR, fluorescence and NMR data has been ascribed by Oldfield et al. to the difference in the timescale of the motion required to average the results obtained using the three techniques. Conventional ESR and fluorescence probe measurements are both unaffected by motion which is slower than approx. 10^8 Hz. Thus it is argued that the lipid disorder seen by ^{2}H-NMR is due to motion in the frequency region intermediate between the NMR and ESR timescale which corresponds to frequencies between 10^5 and 10^8 Hz. This interpretation is still not sufficiently general to apply to all lipid-protein interactions as it has recently been shown by both ^{13}C- and ^{31}P-NMR of glycophorin-phospholipid dispersions that at least two well resolved components are present in the spectrum [16–18]. One of these reflects a strong immobilisation of the phospholipid and is thought to be associated with lipid adjacent to the protein. The other component is very similar to that derived from a lipid in the absence of protein. This shows that the exchange rate of the lipid between the boundary and bulk phases must be slower than 10^3 to 10^4 s^{-1} which is the order of magnitude of the ^{13}C and ^{31}P chemical shift anisotropy. The low-frequency disorder seen for other lipid-protein dispersions is absent.

The NMR linewidth data is ambiguous in the sense that a reduction in linewidth may result from an increase in the molecular mobility of the sample or a conformational change in the molecule containing the resonant nucleus and does not necessarily convey any information on the timescale of the molecular motion other than that it is more rapid than the rigid lattice NMR splitting. We found (Cornell and Keniry, unpublished) that despite their opposing effects on the static chemical shift anisotropy of the ^{13}C resonance of the phospholipid carbonyl groups, gramicidin A^+ and cholesterol both cause a dramatic reduction in $T_{1\rho}$ of the methylene chain protons. We have also shown that the $T_{1\rho}$ of chain protons in the membranes of myelin and whole chloroplasts is between one and two orders of magnitude shorter than that of a fluid phospholipid bilayer [20]. In the present publication we show that the reduction in $T_{1\rho}$ due to adding cholesterol or gramicidin A^+ to a phospholipid bilayer is far more dramatic than the change produced in the laboratory frame spin-lattice relaxation time, T_1, of the same protons. Furthermore it is shown that $T_{1\rho}$ is significantly shorter in erythrocyte membranes than in a bilayer prepared from their lipid extracts whereas T_1 is nearly the same in both cases. Both the erythrocyte membrane cholesterol and protein were found to contribute to the reduction in $T_{1\rho}$.

The significance of T_1 and $T_{1\rho}$ is described more fully in the next section. Briefly, however, a reduction in $T_{1\rho}$ not matched by a similar reduction in T_1 indicates the introduction of a motion within the membrane at relatively low tumbling rates in the region of 10^5 Hz which was not present to the same extent in the pure lipid bilayer. Because of the way in which the superimposed resonances derived from all of the methylene groups within the phospholipid chain, including both the single and double bond carbons are influenced in the same manner we suggest that the low frequency motion introduced by the addition of non-lipid components involves a collective motion of a number of methylene groups. This leads us to speculate that the low frequency motion may possess a greater co-operativity and directly influence a greater number of the surrounding lipids than does the higher frequency motion measured by T_1.

A number of reports are available on the presence of low frequency motions within pure lipid membranes [22–25]. Supporting evidence for an

increase in the intensity of these low-frequency motions in the presence of proteins is available in the recent publications of Kang et al. [26] and Paddy et al. [27]. Supporting evidence that this class of motion is restricted to the lower frequencies is seen in the insensitivity of T_1 recorded for the methylene chain resonances of phospholipids, before and after reconstitution with the Ca^{2+}-pump protein of sarcoplasmic reticulum [28]. To date the low-frequency end of the frequency distribution of motion within membranes has received relatively little attention. Low-frequency motion within membranes could have a significant role to play in information and energy transfer within biological membranes.

Theory

Nuclear magnetic resonance relaxation time measurements provide a method of determining the frequency distribution of molecular motion. By employing a variety of NMR techniques it is possible to measure reorientation rates over a range of values from less than 10^{-1} to more than 10^{10} Hz.

The relationship between the T_1 and $T_{1\rho}$ relaxation times and the intensity of motion $I(\omega)$ at frequency ω is given by [21]

$$T_1^{-1} \approx K_1 I(\omega_0)$$

$$T_{1\rho}^{-1} \approx K_2 I(2\omega_1)$$

where T_1 and $T_{1\rho}$ are the spin-lattice relaxation times in the laboratory and rotating reference frames, and ω_0 and ω_1 are the precession frequencies appropriate to the main magnetic field and the rotating transmitter field. In the present case $\omega_0/2\pi \approx 10^8$ Hz and $2\omega_1/2\pi \approx 10^5$ Hz. K_1 and K_2 are constants dependent on the type of motion undergone by the group containing the resonant nuclei.

A schematic representation of the frequency distribution of motion for a methylene group within a bilayer membrane is shown in Fig. 1, [22]. The horizontal axis is drawn as a logarithmic scale of reorientation rate. The intensity of motion at a particular frequency is taken as the vertical axis. As shown in Fig. 1, $T_{1\rho}^{-1}$ is approximately proportional to the intensity of motion near 10^5 Hz, and T_1^{-1} to the intensity near 10^8 Hz. The usual form of the frequency distribution in liquid systems is a monotonic decay towards higher frequencies. This behaviour is a consequence of the random motion undergone by the molecules in a liquid. Over the frequency scale accessible to NMR it is extremely uncommon to observe a peak in the distribution function. Such a peak would indicate an increase in the intensity of motion over a narrow range of molecular reorientation rates, as could result from a mechanical vibration. Such resonant forms of molecular motion are not usually detected until far higher frequencies in the infra-red region of the electromagnetic spectrum.

Since the only source of energy for motion is thermal energy, for a given temperature, the integrated area beneath the curve in Fig. 1 must always remain constant. Because the frequency range covered by motion exceeds 10^{10} Hz, any change in the frequency distribution at lower frequencies near 10^5 Hz causes a negligible change in the redistribution of the area beneath the curve. Thus whereas changes at the high-frequency end of the frequency scale will influence both T_1 and $T_{1\rho}$, changes in the low-frequency intensity predominantly influence $T_{1\rho}$. Thus it is necessary to measure both $T_{1\rho}$ and T_1 to establish whether a change in $T_{1\rho}$ reflects a change in the intensity of a very high frequency motion near or above 10^{10} Hz or a genuine increase in the intensity of the low-frequency end of the spectrum.

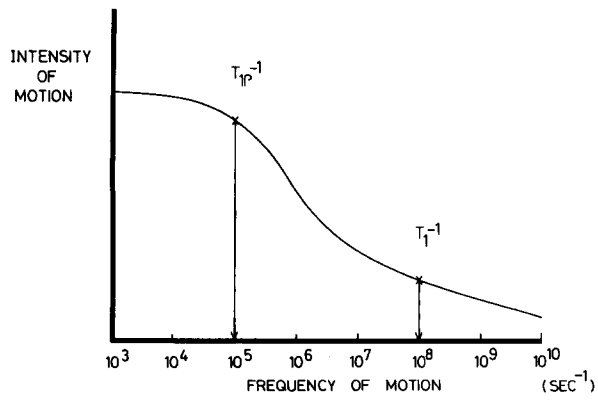


Fig. 1. A schematic representation of the frequency distribution for the methylene protons of a lipid hydrocarbon chain. The regions influencing the spin-lattice relaxation times (T_1) and the spin-lattice relaxation time in the rotating reference frame ($T_{1\rho}$) are shown.

A quantitative description of a particular class of motion is described by the highest frequency of reorientation associated with that motion or alternatively its inverse, known as the correlation time t_c.

Materials and Methods

Human packed red blood cells were obtained from the Red Cross Transfusion Service, Sydney. Erythrocyte membranes were prepared by the method of Ralston [30] and the spectrin removed by incubating in distilled water at 37°C for 15 min. The despectrinated ghosts were washed twice in water or 0.1 M EDTA and pelleted at $50000 \times g$ for 30 min. Approximately one-fifth of each preparation was used as whole ghosts and the remainder washed in distilled water and lyophilised overnight. The lyophilised material was used to prepare lipid extracts of the erythrocyte membranes. A total lipid extract was obtained using the method of Bligh and Dyer [31] with 50 mM EDTA and 100 mM NaCl in the aqueous phase. A cholesterol-free fraction of the total lipid extract was collected by preparative TLC on silica gel (Merck) preparative TLC plates and developed with $CHCl_3/CH_3OH/H_2O$ (65:25:4, v/v). The lipid bands were revealed by spraying their surface with glass distilled water. The cholesterol band was removed and the remaining lipids reclaimed by washing the gel with $CHCl_3/CH_3OH$. Egg yolk phosphatidylcholine (egg PC), cholesterol and dimyristoylphosphatidylcholine (DMPC) were obtained from Calbiochem (La Jolla U.S.A.) and used without further purification. The purity of the egg PC and DMPC were confirmed both prior to and following their examination by NMR using TLC as described above. All of the lipid dispersions were hydrated with an equal weight of H_2O. The erythrocyte membrane pellets were found to be approximately 20:1 by weight of H_2O.

Gramicidin 'pure' was purchased from Koch Light U.K. and shown by amino acid analysis to consist of 75% gramicidin A and 25% of other linear gramicidins B and C. Natural mixtures of closely related linear gramicidins, rich in gramicidin A have been denoted gramicidin A^+ [29], which is the nomenclature we have followed here.

In order to measure the $T_{1\rho}$ of the sample protons we have used the ^{13}C cross polarization technique [32]. This approach has the advantage that the $T_{1\rho}$ may be obtained for several populations of protons which are normally unresolved by conventional ^1H-NMR spectroscopy. The principle of the technique is that magnetic order is first produced for the abundant proton population within the sample and then transfered to the relatively rare ^{13}C nuclei to which they are magnetically coupled by the carbon-proton dipolar interaction. The contact between the two spin systems is facilitated by simultaneously irradiating the sample with rotating radio frequency magnetic fields tuned to both the ^{13}C and ^1H resonance frequencies. The intensity of the carbon and proton irradiation fields were adjusted to provide the best signal-to-noise ratio for the carbon spectrum following the cessation of the carbon irradiation. By following the growth and decay of the ^{13}C spectral amplitude for a variety of contact times, it is possible to deduce the rate of carbon-proton cross polarization $(T_{C-H})^{-1}$ and the return to equilibrium of the protonic magnetisation $(T_{1\rho})^{-1}$. Should these processes occur exponentially, one may define the rate constants from the amplitude (Y) of the ^{13}C spectra by [32]

$$Y = \exp(-t_m/T_{1\rho})[1 - \exp(-t_m/T_{C-H})] \qquad (1)$$

and t_m is the contact time between the carbon and proton populations.

It is assumed here that the intrinsic $T_{1\rho}$ and T_1 of the ^{13}C nuclei are sufficiently longer than the proton relaxation times to permit the approximation that the relaxation is dominated by the changes in magnetisation of the proton population. The advantage of measuring proton relaxation times is that they are influenced by a wide range of motions including both intra-molecular reorientations and fluctuations in inter-molecular chain spacing. Further studies are in progress in which the intrinsic ^{13}C $T_{1\rho}$ is being measured using the same general technique. The intrinsic ^{13}C $T_{1\rho}$ is dominated by the intra-molecular carbon-proton dipolar interactions and promises to provide further information on the details of these low frequency motions.

By using a minor modification of the conventional proton enhanced pulse sequence it is also

possible to measure the T_1 spin-lattice relaxation time for the protons effective in cross polarization. The technique involves inverting the proton population a time t prior to performing the proton enhanced pulse sequence and accumulating the ^{13}C spectra. By measuring the amplitude of the ^{13}C spectrum as a function of t, the recovery of the proton magnetisation may be determined.

The ratio of $T_{1\rho}$ to T_1 for the protons involved in cross polarization has been taken here as the measure of the relative intensity of low to high frequency motion undergone by the group under study.

All spectra were obtained using a Bruker CXP100 spectrometer operating at 22.63 MHz for ^{13}C and 90.02 MHz for ^{1}H. The magnet was stabilized using an external ^{2}H lock signal. Typical spectrometer conditions are given in the caption to Fig. 2(c).

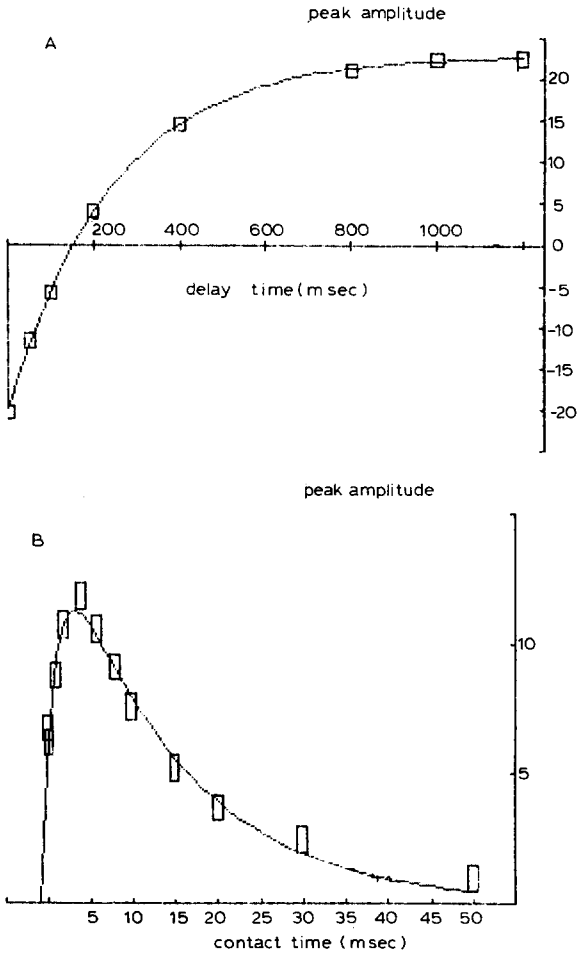
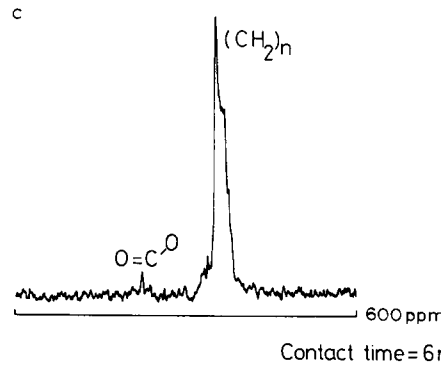


Fig. 2. Examples of the relaxation functions from which the estimates of T_1 and $T_{1\rho}$ shown in Table I were obtained.
(a) The laboratory frame spin-lattice relaxation function obtained using the modified ^{13}C proton enhanced (PE) sequence for DMPC/cholesterol (1.7 : 1 molar ratio) in water with a lipid mixture/water ratio of 1 : 1 (w/w). The main methylene peak derived from spectra similar to the one shown in Fig. 2c was plotted for a range of proton channel inversion delays, t, (see text). The continuous curve fitted to the data is a single exponential from which T_1 was obtained.
(b) The rotating frame spin-lattice relaxation function obtained using a conventional PE pulse sequence [32], for DMPC/cholesterol (1.7 : 1 molar ratio) in water with a lipid mixture/water ratio of 1 : 1 (w/w). The main methylene peak amplitude was recorded for a range of contact times (see text). The continuous curve fitted to the data points correspond to a double exponential increase and a single exponential decay from which $T_{1\rho}$ was obtained.

Results

Figs. 2a and b, 3a and b and 4a and b show examples of the change in amplitude of the methylene peak in the ^{13}C spectrum of a number of different lipid dispersions during modified and conventional proton enhanced pulse sequences. In the conventional sequence the signal growth and decay was tested against expressions of the form shown in Eqn. 1. In general, it was not possible to



(c) An example of the spectral quality of ^{13}C PE spectra from which the peak amplitudes shown in Figs. 2 (a and b) were obtained. All spectra were obtained using a Bruker CXP100 spectrometer operating at 22.63 MHz for ^{13}C and 90.02 MHz for protons. The spectrometer conditions were typically sweep width :20000 Hz; linebroadening :35 Hz; duration of spin locking pulse :9 μs; acquisition time : 20 ms; pulse repetition delay : 1 s; number of acquisitions per point :3600; the contact times for the T_1 measurements are shown on the individual graphs shown here and in general were between 1–10 ms.

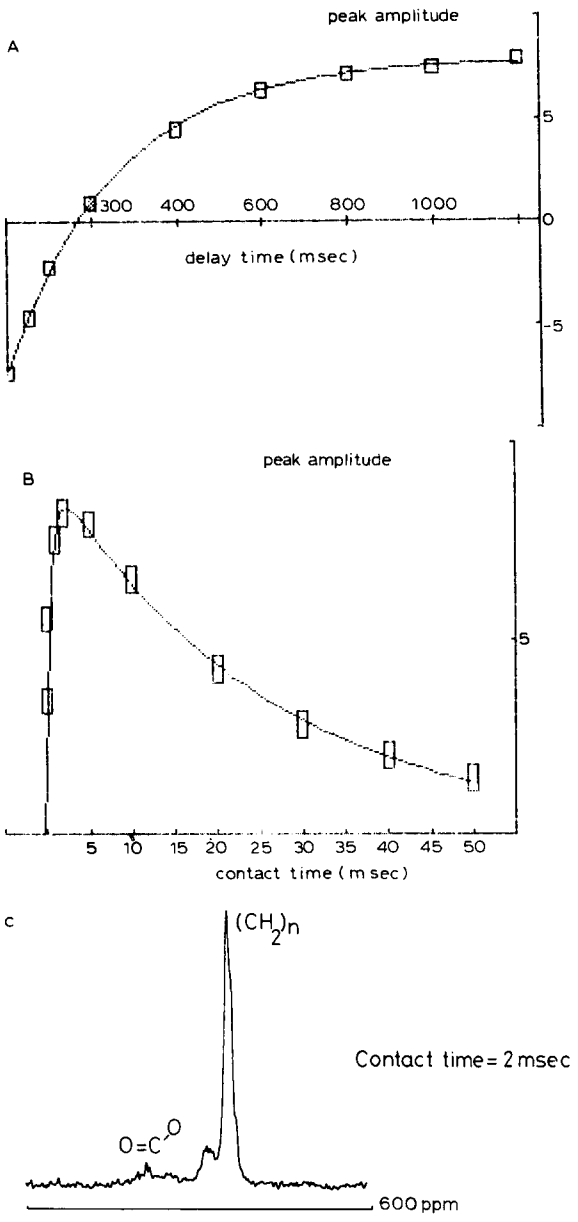


Fig. 3. (a) The laboratory frame spin-lattice relaxation function obtained using the modified ^{13}C PE sequence for DMPC/gramicidin A^+ (4:1 molar ratio) in water with a lipid mixture/water ratio of 1:1 (w/w). The same procedure was used as described in Fig. 2a.
(b) The rotating frame spin-lattice relaxation function obtained using the conventional PE sequence as described in Fig. 2b. For DMPC/gramicidin A^+ (4:1 molar ratio) in water with a lipid mixture/water ratio of 1:1 (w/w).
(c) An example of the spectral quality of the ^{13}C PE spectra from which the peak amplitudes shown in Figs. 3 (a and b) were obtained. The spectrometer conditions were the same as shown for Fig. 2.

fit the cross polarization region of the data at short time to a single exponential increase. At longer times in the region dominated by the rotating frame spin lattice relaxation processes the signal decay was found to obey the $\exp(-t_m/T_{1\rho})$ part of Eqn. 1. The continuous curves drawn through the data points in Figs. 2b, 3b and 4b represent a minimised absolute differences computer fit of a double exponential increase and single exponential decay to the intensity data. The choice of the double exponential increase for the intensity data is not claimed to be unique, and is used here simply in order to obtain a better estimate of the $T_{1\rho}$ portion of the curve.

The spectral inversion resulting from the application of the modified proton enhanced pulse sequence could always be fitted to an exponential function within the accuracy of the experimental results. Examples of the agreement between the data and the exponential inversion are shown in Figs. 2a, 3a and 4a. In Table I we have summarised the $T_{1\rho}$ and T_1 relaxation times obtained from these procedures for the various dispersions studied here. The uncertainties denote the scatter derived from at least three different sample preparations. Care was taken to prevent radio frequency heating of the sample by the transmitter and decoupler irradiation. Such heating can cause $T_{1\rho}$ to lengthen, reducing the ratio of $T_1/T_{1\rho}$.

Previous measurements of $T_{1\rho}$ of protons in aligned egg PC has shown $T_{1\rho}$ to be anisotropic and to depend upon the orientation of the long axis of the phosphatidylcholine hydrocarbon chain relative to the external magnetic field [22]. The detailed form of the angular dependence is at present unclear. Other studies on coarse lipid dispersions of phospholipid have described the spin lattice relaxation in the rotating reference frame in terms of a single exponential decay constant [33]. The continuous distribution of decay constants in a coarse lipid dispersion caused by the orientation dependence of $T_{1\rho}$ produces a relaxation function which is difficult to distinguish from a single exponential. The possibility therefore exists that the single exponential functions fitted here are actually being forced onto a complex decay curve composed of many exponentials. However the magnitude of the effects we are reporting here show that the qualitative conclusions we are mak-

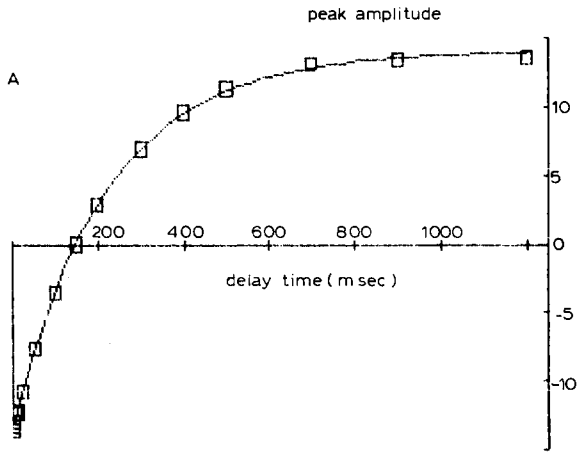
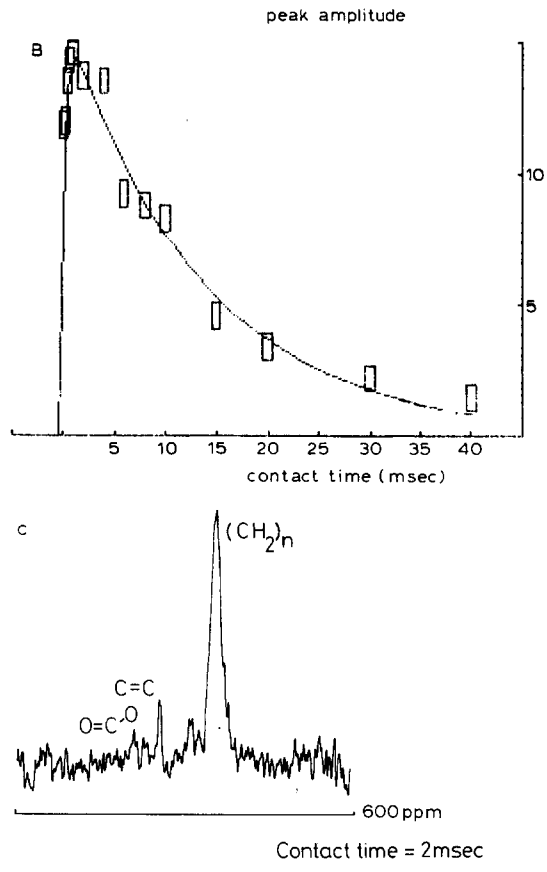


Fig. 4. (a) The laboratory frame spin-lattice relaxation function obtained using the modified ^{13}C PE sequence for despectrinated erythrocyte membranes (see Materials and Methods) obtained as described in the caption to Fig. 2a.



(b) The rotating frame spin-lattice relaxation function obtained using the conventional PE sequence for despectrinated erythrocyte membranes (see Materials and Methods) obtained as described in the caption to Fig. 2b.
(c) An example of the spectral quality of the ^{13}C PE spectra from which the peak amplitude shown in Figs. 4 (a and b) were obtained. The spectrometer operating conditions were the same as for Fig. 2.

ing from these data are not altered by this possibility. For convenience we have also shown the ratio of $T_1/T_{1\rho}$ for each of the cases studied here. The increase in this ratio caused by the addition of cholesterol or non-lipid components to the dispersions is dominated by the reduction in $T_{1\rho}$. Whereas $T_{1\rho}$ has a range of values from 12 ms to 85 ms, a change of over 700%, T_1 varies from 195 ms to 250 ms which is a variation of only 25%. The results obtained from the erythrocyte membranes and their lipid extracts show more than a 2-fold decrease in the $T_1/T_{1\rho}$ ratio on extracting the lipid-cholesterol fraction from the membrane and a further reduction upon removing the cholesterol. Comparing the ratio obtained for the cholesterol

TABLE I

THE RELAXATION TIMES T_1, $T_{1\rho}$ AND THEIR RATIO FOR A VARIETY OF LIPID AND LIPID-PROTEIN SYSTEMS.

All measurements were made at 305 K.

	$T_{1\rho}$ (ms)	T_1 (ms)	$T_1/T_{1\rho}$
DMPC	85±10	210±20	2.5
Egg PC	55±5	220±5	4.0
Cholesterol-free lipid extract of erythrocyte membranes	55±5	220±15	4.0
Total lipid extract of erythrocyte membranes	33±3	200±20	6.1
DMPC/gramicidin A^+ 4:1, molar ratio	26±3	250±10	9.6
DMPC/cholesterol 1.7:1, molar ratio	14±3	242±10	16.6
Erythrocyte membranes in EDTA	12±3	195±20	16.3

free lipid extract if the erythrocyte membranes with that of egg PC suggests that the behaviour of the cholesterol-free erythrocyte membrane lipid is very similar to a phosphatidylcholine in the fluid L_α phase and that the additional heterogeneity of lipid class and chainlength in the erythrocyte membranes does not contribute any additional effects.

Of particular interest is the absence of any clear two-component decay of magnetisation in the systems studied here. This was true for both the measurements of $T_{1\rho}$ and T_1. Although the double resonance technique can average multiple relaxation behaviour due to spin diffusion within the proton population the results are consistent with the lipids undergoing a rapid exchange between all of the sites available to them during the 10^{-3} to 10^{-4} s duration of the experiment. Since $T_{1\rho}$ is in the region 12–85 ms it is unlikely that spin diffusion is sufficiently rapid to contribute to this apparent exchange.

Discussion

In the present study the inclusion of high concentrations of cholesterol, polypeptides or proteins caused a far greater effect in shortening $T_{1\rho}$ than T_1. This shows that the intensity of motion at frequencies near 10^5 Hz has been increased relative to frequencies near 10^8 Hz. The insensitivity of T_1 shows that this effect is not simply a result of a high frequency motion above 10^8 Hz being slowed down. The addition of protein causes the introduction of a motion not previously present within the membrane. This may either be a new class of motion or the enhancement of an existing although previously low amplitude motion of the lipid hydrocarbon chains. Motion at these low frequencies has been observed in pure lipid dispersions [22–25]. However when the ratio of $T_1/T_{1\rho}$ is less than 3 it is necessary to make a more complete measure of the frequency distribution of the motion than obtained here to show conclusively the existence of correlation times in the region of 10^{-5} s [34,35]. In the limit of rapid motion such that all of the correlation times for the group under study are shorter than ω_1^{-1} or ω_0^{-1} then the expected limit for $T_1/T_{1\rho}$ is in the range 2 to 3 [34,35].

The total kinetic energy of these low-frequency motions is very small when compared to the total kinetic energy of the lipid chain. For example should a motion with a correlation time near 10^{-8} s be eliminated and its energy converted totally to a new type of motion with a correlation time of 10^{-5} s a 1% reduction in total energy of motion up to frequencies of 10^8 Hz results in a 10-fold increase in the intensity of motion up to 10^5 Hz. Thus it can be seen that $T_{1\rho}$ is far more sensitive to low energy events within the membrane than is T_1.

The greater sensitivity of $T_{1\rho}$ may also be influenced by the lower-frequency motion propagating a greater distance from the lipid-protein interface than does the motion affecting T_1. Since T_1 is thought to depend to a large extent on intra-chain carbon-carbon bond rotations [34], should these only be influenced when the lipid is immediately adjacent to a protein [4] then the effect of the protein is lessened by the averaging of the T_1 for these lipids with those elsewhere in the dispersion away from the protein. However should the low-frequency motion be influenced by the protein at a distance greater than the nearest neighbour or boundary layer of lipid then this reduction due to averaging is less important as many more lipids experience the same effects.

Since the $T_1/T_{1\rho}$ ratio for the superimposed resonance peaks derived from all parts of the lipid chain is shorter in the presence of the membrane protein than in the protein free extracts, the chain motion influencing $T_{1\rho}$ is likely to be a collective movement of many methylene groups as a rigid rod rather than an intra molecular bond rotation. A collective motion is also consistent with the relatively long correlation times which must be present to influence $T_{1\rho}$ preferentially. The relaxation times may be averaged to some extent by spin diffusion between the adjacent methylene groups along the hydrocarbon chain. However as mentioned above the effects of spin diffusion will be relatively short range over the 12–85 ms duration of $T_{1\rho}$. The value of the effective diffusion constant 'D' due to spin diffusion is typically 10^{-13} cm^2/s [36]. Thus during the period of a 12 ms $T_{1\rho}$ spin diffusion would average the signals obtained over a length of hydrocarbon chain approx. 0.5 nm long. By comparing the results obtained here for the synthetic saturated DMPC, the unsaturated egg PC and the lipid extracts of the erythrocyte

membranes it is clear that the low frequency motion due to the presence of protein is far more intense than that produced by mixing saturated and unsaturated lipid chains or by a heterogeneity of lipid class. Thus the low frequency behaviour in the erythrocyte membranes is seen as a result of the protein and to a lesser extent, the cholesterol, being present in the membrane.

Summarising we may thus conclude that lipid-cholesterol, lipid-polypeptide and lipid-protein dispersions examined here cause very little attenuation in the hydrocarbon chain bond rotations and kinks which contribute to T_1. Short range interactions of this nature may occur immediately adjacent to a protein surface. However these effects are averaged by rapid molecular exchange with the total lipid population elsewhere within the system. The major effect arising from the lipid-protein interactions is longer range fluctuations of order in the form of either a co-operative swaying motion of large segments of the lipid chains or co-operative fluctuations in the local chain density.

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