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A ^{13}C NMR SPIN-LATTICE RELAXATION STUDY OF THE INTERACTION OF MYELIN PROTEINS WITH LIPID VESICLES

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Natural abundance ^{13}C nuclear magnetic spin-lattice relaxation times have been measured for bovine brain phosphatidylserine vesicles with and without bound proteins. The relaxation times were lower than published values for the corresponding nuclei in egg phosphatidylcholine, but showed the same trend, with relaxation times increasing along the acyl chains away from the polar headgroup. These times were inversely related to the degree of saturation of the lipid. Cytochrome c caused insignificant changes in the lipid acyl chain relaxation rates but reduced the resonance intensities, in agreement with Brown and Wüthrich (Biochem. Biophys. Acta 468 (1977) 389). In contrast, the basic protein from bovine myelin did not affect the intensities but reduced the relaxation times for ^{13}C nuclei near the bilayer centre, and for nuclei near carbon-carbon double bonds. These proteins also dramatically broadened the serine headgroup carboxyl resonance. It appears, in accord with other recent evidence, that the basic protein does penetrate the hydrophobic region of the bilayer (possibly to the centre), producing quantitatively similar changes in the relaxation rates to proteolipid protein, an integral membrane protein.

1. Introduction

Considerable use has been made of ^{13}C NMR in the study of the dynamic structures of phospholipid bilayers and cell membranes [1–5]. This technique does not require the use of probe molecules and is thus not subject to the interpretative difficulties associated with esr studies employing spin-labelled molecules [6,7]. The chemical shift range is large (about 200 ppm) resulting in resolution of several of the methylene groups within the extended acyl chains of lipid molecules. Magnetic relaxation is primarily through *intra*-molecular dipole–dipole interactions for protonated carbon atoms, providing a simpler interpretation of relaxation times in terms of nuclear motion than is generally available from ^{1}H NMR data.

From ^{1}H, ^{2}H and ^{13}C NMR studies of synthetic and natural lipid bilayer membranes a description of the mobility and order of lipid molecules within membranes has emerged. Analysis of the relaxation rates in liquid crystalline arrays of lipids is complicated by the anisotropic motion of the nuclei: the relaxation times of each nucleus are modulated by vesicular rotation, molecular translational motion within the bilayer, molecular rotation, and intramolecular rotational isomerization. The interpretative difficulties have been marked particularly by the controversy over the substantial differences in ^{1}H and ^{13}C NMR linewidths for single bilayer and multilamellar vesicles. Some consensus has however been reached about the motions governing spin-lattice relaxation rates of ^{13}C nuclei in lipids, which are very similar in uni- and multilamellar vesicles, allowing cautious interpretation of changes in terms of perturbation of molecular motion and order [8–10].

A full description of the motion of lipid acyl groups requires relaxation rates for each nucleus in the chain. This would necessitate use of a series of ^{13}C-enriched lipids as in the spectra obtained at natural abundance many of the resonances are unresolved. But measurements on unenriched lipids do allow some semi-quan-

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Abbreviations used: PC, phosphatidylcholine; PS, phosphatidylserine; PLP, proteolipid protein; MBP, myelin basic protein; NMR, nuclear magnetic resonance.

titative deductions which may also serve to guide the design of subsequent experiments.

Studies of the effects of added molecules on the mobility of lipid molecules in bilayers are limited, but include observations on the effects of cholesterol [2, 11] and some proteins [12–15]. We report here the effects of two central nervous sytem proteins, the proteolipid protein and the basic protein, on bilayer structure. The former appears to be an intrinsic membrane protein whereas the latter is generally accepted as being extrinsic to the myelin membrane. As noted in earlier papers, and discussed more fully below, some recent observations have in contrast suggested at least partial penetration of the basic protein into the hydrophobic domain of monolayers and bilayers. We have attempted to monitor more directly this intercalation into bilayers by following the effect of the protein on lipid acyl chain mobility, and by comparison with the corresponding effects of the proteolipid protein and cytochrome c.

2. Methods

L-α-phosphatidylserine (PS) was purified from bovine brain extract Type III [16] (Sigma, St. Louis, USA) by column chromatography on DEAE-cellulose [17]. It was tested for purity on thin layer silica gel plates using $CHCl_3/CH_3OH/CH_3CO_2H/H_2O$ (25 : 15 : 4 : 2, by volume). The lipids were detected by charring with sulphuric acid, or by a specific phosphate stain [18]. Impurities were estimated to be less than 1%. PS was stored at −20°C under N_2 in $CHCl_3/CH_3OH$ (3 : 1, by volume). It was prepared only as required and rarely kept longer than 3 months.

Vesicles were prepared at 4 ± 1°C under N_2 in a deuterated 5 mM Tris/1 mM EDTA buffer at pD 9.5 using an ultrasonic bath. Undispersed lipids were removed by centrifugation at 10 000 r.p.m. for 10 min in an SW50.1 rotor (Beckman, Palo Alto, California).

Myelin basic protein (MBP) was prepared from bovine brain as described elsewhere [19]. Crystalline horse heart cytochrome c was purchased from Calbiochem (California, USA) and used without further purification. The proteolipid protein of myelin (PLP) was prepared from myelin by a method similar to that of Reynolds and Green [20]. The myelin was treated with acidified $CHCl_3/CH_3OH$ (2 : 1, by volume) to precipitate MBP [20]. The organic solvent was removed from the supernatant and the residue taken up in 0.1 M Tris, 10% sodium dodecyl sulphate, 0.1 M NaCl buffer. The protein was then applied to a Sepharose 4B column equilibrated with 0.1 M Tris, 0.3% sodium dodecyl sulphate, 0.1 M NaCl buffer. Before application, dithiothreitol (1 mg/15 mg of dry myelin) was added to the sample solution. The protein peak was collected, concentrated and dialyzed and then rechromatographed. SDS polyacrylamide gel electrophoresis of the protein revealed the presence of no basic protein.

The protein-lipid complexes for MBP and cytochrome c were formed by adding a small volume of concentrated protein solution (100–200 mg/ml) to the lipid dispersion after sonication. In a typical experiment 300 μℓ of a 200 mg/ml solution of protein in 5 mM Tris/1 mM EDTA pD 9.5 buffer was added to the dispersion (3 mℓ, 100 mg/mℓ). The PLP-lipid complex was formed by mixing a dispersion of PLP in a cholate buffer. In a typical experiment 30 mg of PLP in 2 mℓ of 1% cholate/5 mM Tris/1 mM EDTA pH 9.5 buffer was combined and vortexed with 150 mg of PS dispersed in 5 mℓ of the same buffer. The mixture was then dialyzed for 24 hrs at 4°C under N_2 against four 5 litre washes of 5 mM Tris/1 mM EDTA pH 9.5 buffer. Then solution was then concentrated on an XM300 ultrafiltration membrane (Amicon, Lexington, USA) and the buffer exchanged with 5 mM Tris/1 mM EDTA pD 9.5 D_2O buffer and finally sonicated for 20 min. A control experiment was also performed in which PS in 1% cholate/5 mM Tris/1 mM EDTA pH 9.5 was dialyzed, concentrated and sonicated as set out above. Typically in the experiments above the protein : lipid ratio was 1 : 5 (g/g). Phosphorus analyses and the determination of bound protein are described elsewhere [19]. Analyses of the lipid by thin-layer chromatography after the experiment revealed minimal degradation. The integrity of the MBP after the completion of the relaxation time experiment was checked by SDS polyacrylamide electrophoresis; no degradation was observed (MBP has previously been observed to break down under basic conditions when no lipids are present [21]). One T_1 experiment (for PLP bound to PS) was repeated immediately after the first experiment to check the reproducibility of the data.

^{13}C NMR spectra were obtained with 10 mm and 15 mm tubes, at 22.625 MHz on a Bruker HX-90 spectrometer fitted with quadrature detection. Protons were decoupled from carbon with a Bruker broadband decoupler. Chemical shifts were referenced to an external capillary of tetramethylsilane. The spectral width was 5000 Hz and 1 Hz digital broadening and 8192 time-domain addresses were used.

^{13}C T_1 measurements were performed using the FIRFT method of Canet et al. [22] utilizing a waiting time of 1.319 s and from 8 to 10 τ values. T_1 values were calculated from the experimental peak amplitudes both semilogarithmically and exponentially by the method of Sass and Zeissow [23]. In some later experiments the method of Hanssum et al. [24] was used. Area measurements were made by calculating the area of a lorentzian line using the experimentally determined height and line width. The areas were normalized assuming an area ratio of 1.0 for an external capillary of tetramethylsilane doped with diphenylpicrylhydrazyl.

3. Results

The MBP-PS and cytochrome c-PS dispersions were formed by adding a concentrated protein solution to a dispersion of preformed vesicles except in one experiment where the basic protein was co-sonicated with the lipid vesicles. The latter experiment was carried out several times and on all but one occasion the aggregation of the vesicles was so severe no relaxation time measurements could be made. Spectrum C in fig. 2 represents the most successful of these attempts. The same effect was noticed in an earlier study when it was attempted to incorporate protein on both sides of the bilayer [25]. In all of the above experiments it was found that fusion and aggregation on incorporation of MBP at the lipid concentrations required for a ^{13}C relaxation time study could be minimized by performing the experiment at pD 9.5 at low ionic strength and forming the protein-lipid complex by adding protein to the preformed vesicles.

When PLP was complexed to PS either by dialysis of cholate solutions or 2-chloroethanol solutions no aggregation of the lipid vesicles was noticed. Similarly no aggregation was noticed on adding cytochrome c. Although aggregation and fusion lead to spectral broadening, several studies [8,9,26] have shown that ^{13}C longitudinal relaxation times are unaffected by vesicle size. In the above experiments the amount of MBP bound to the lipids varied from 60–80%, and 86% of the cytochrome c added was found to be bound to the lipid. Circular dichroism spectra were also run after the relaxation time experiments and the conformation of MBP was found to have changed from its unstructured form to a conformation possessing 20% α-helix when bound to PS [19].

In all the spectra there appears to be no differential broadening of any of the peaks except that both MBP and PLP broaden the serine carboxyl resonance to such an extent that it is barely visible above the noise. This is an indication that both proteins may interact strongly with the serine carboxylate.

4. Resonance assignment

A typical ^{13}C spectrum of phosphatidylserine prepared as an aqueous solution of bilayer vesicles is given in fig. 1. Most aspects of the spectrum are identical with the spectrum of egg yolk lecithin, which has been reported by several workers [4,27,28] except, as expected, in the head group region. Head group resonances were assigned by comparison with the resonances of model compounds such as serine and phosphoserine [29] (table 1).

Of the two upfield resonances at 175.4 ppm and 173.0 ppm, the resonance at 175.4 ppm corresponds to the ester carbonyl group observed in other studies of phospholipids. The resonance at 173.0 ppm is tentatively assigned to the serine carboxyl.

5. Intensity measurements

Within experimental error there were no changes in the intensities of the methylene chain resonances or the resonances of the two terminal nuclei when MBP was added to PS vesicles (table 2). This contrasts with the change observed by Brown and Wüthrich [13] on incorporation of cytochrome c into phosphatidylcholine cardiolipin vesicles. We also observed a decrease in the intensity of the methylene envelope compared to the intensity of the resonances of the terminal nuclei of the alkyl chain on addition of cytochrome c to PS

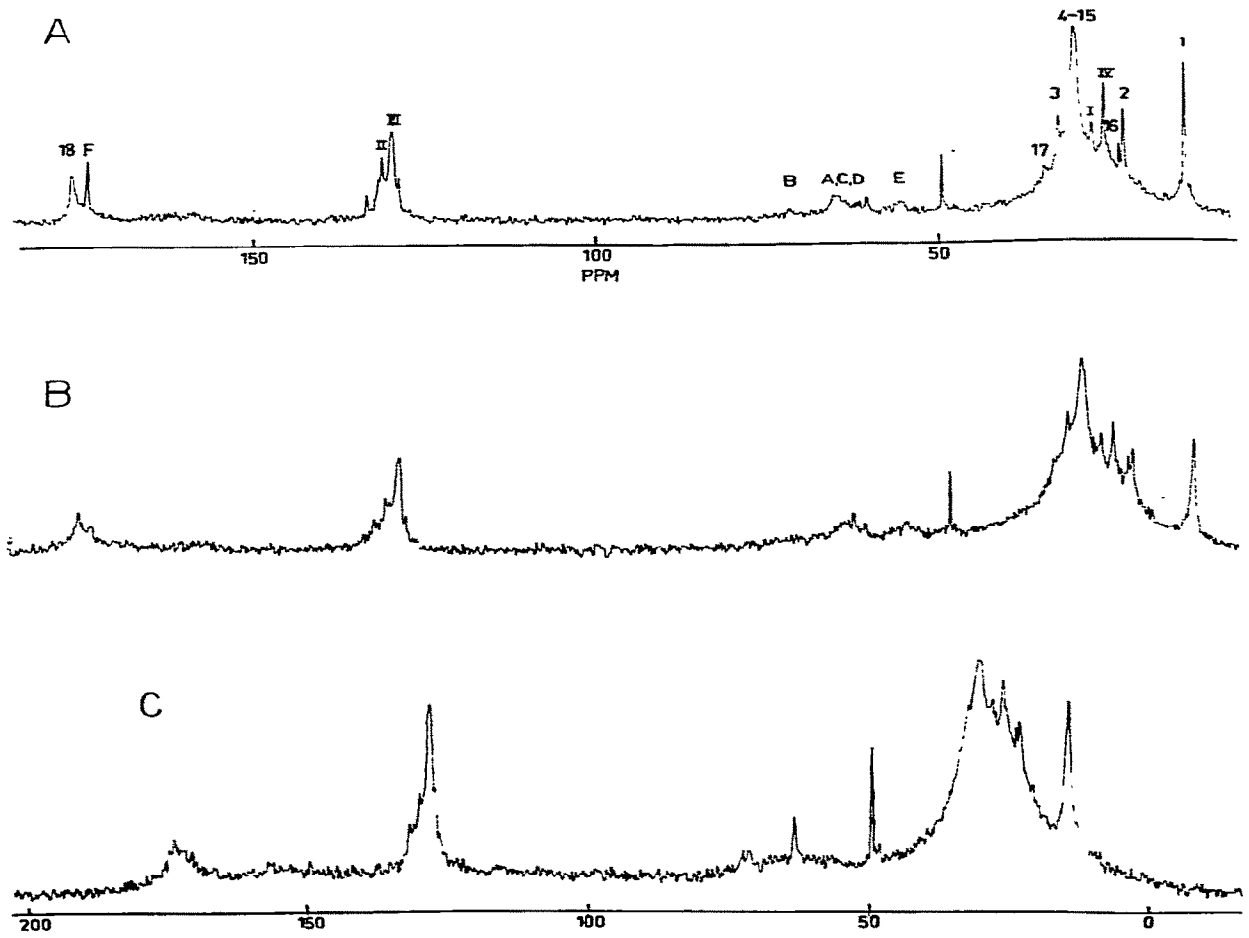

Fig. 1. 22.63 MHz ^{13}C spectra of A) Phosphatidylserine vesicles; B) Phosphatidylserine vesicles with MBP; (1 : 5 g protein/g lipid) added to the aqueous dispersion of PS vesicles; C) Phosphatidylserine vesicles with MBP; (1 : 5 g protein/g lipid) co-sonicated with the lipid prior to measurement. All in a deuterated buffer (5 mM Tris, 1 mM EDTA) at pD 9.5 and 31°C.

vesicles. In accord with the spectra obtained by Brown and Wüthrich [13] no protein resonances were observed for any of the proteins. As previously noted [13], some protein resonances should be observable at the protein to lipid ratios employed, unless broadening has resulted from an increase in the rotational correlation time of the protein on binding to the vesicles.

6. Longitudinal relaxation rates

It has been shown that the dominant spin-lattice relaxation mechanism for ^{13}C nuclei is through the intramolecular dipole-dipole interaction with the directly bonded hydrogens [30,31]. The motion of carbon atoms along the chain in a bilayer is anisotropic and their relaxation times are a function of all the correlation times that describe the motion of the nuclei, as well as the degree of order [5].

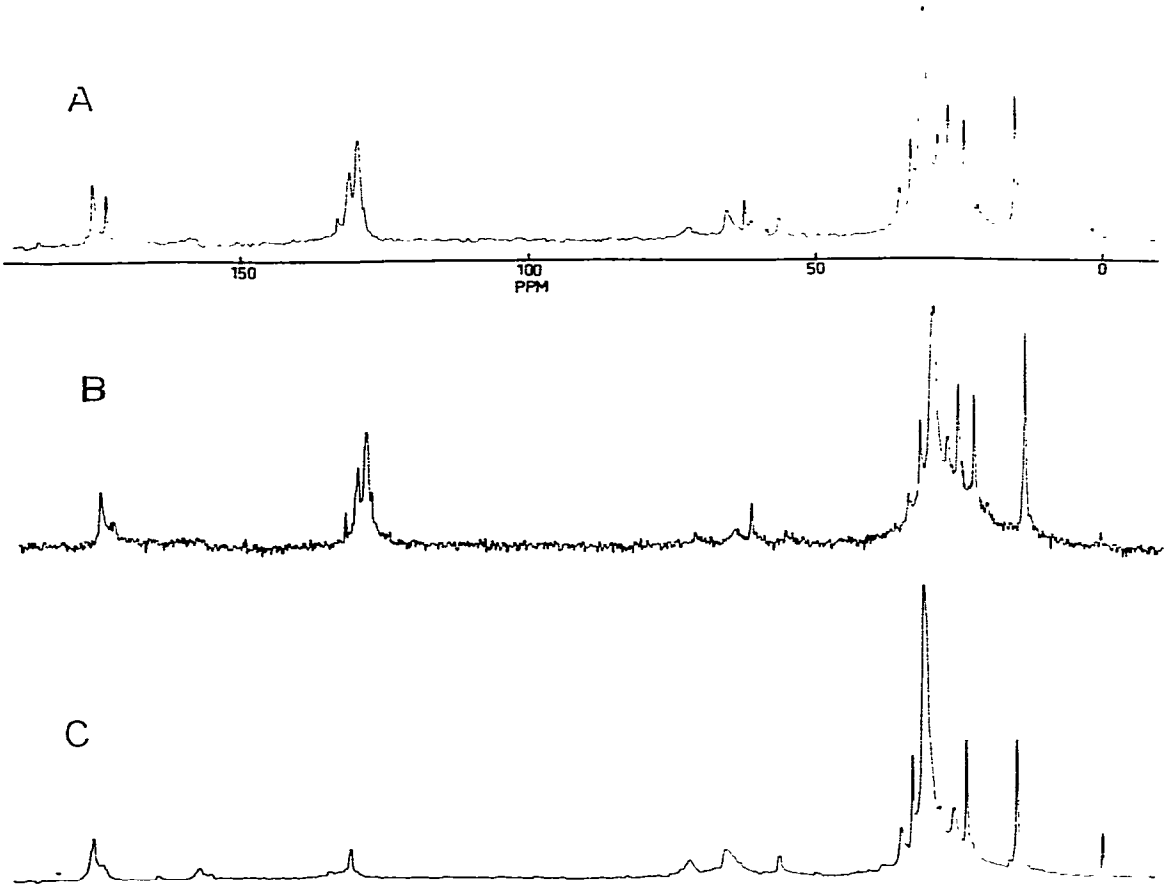

Fig. 2. 22.63 MHz ^{13}C spectra of A) Phosphatidylserine vesicles prepared by dialysis of cholate followed by sonication (see text); B) Phosphatidylserine vesicles with PLP included,(prepared by dialysis of cholate followed by sonication); C) Phosphatidylserine (saturated) vesicles. All in a deuterated buffer (5 mM Tris, 1 mM EDTA) at pD 9.5 and 31°C.

Table 3 sets out the relaxation times for nuclei along the hydrocarbon chain of phosphatidylserine with and without MBP intercalated into the bilayer at pD 9.5. Relaxation times were obtained for each batch of PS prepared, since the degree of unsaturation varied. Not unexpectedly, the relaxation times for PS increase on going from the head group end to the terminal methyl end of the chain. This is in agreement with all previous studies of phospholipid bilayers [2,8,9,10] and is a manifestation of the well-known mobility gradient. On interaction with MBP there are decreases in the relaxation times of the terminal methyl group and the two carbon nuclei closest to this as well as in the unsaturated carbons and the nuclei next to these. But there are no significant changes in the relaxation times of nuclei elsewhere along the chain. In particular, the methylene chain resonance, which is expected to give the most accurate relaxation time, shows no significant decrease.

When MBP was cosonicated with PS (and hence accessible to both sides of the bilayer) the same effect was seen (table 4), although the lower resolution in this case allowed accurate determination of T_1 for only three resonances. A qualitatively similar effect

Table 1
Chemical shift assignments for phosphatidylserine vesicles in a deuterated buffer (5 mM Tris, 1 mM EDTA) at pD 9.5 and 31°C

Number [a]	Assignment	Chemical shift (ppm)
1	t-CH_3	15.1
2	$\underline{C}H_2CH_3$	23.9
3	$\underline{C}H_2CH_2CH_3$	33.2
4–15	$(CH_2)_n$	31.3
16	$\underline{C}H_2CH_2C{=}O$	33.1
17	$\underline{C}H_2C{=}O$	35.1
18	C=O, glycerol	175.4
I	$\underline{C}H_2CH{=}CH$	28.3
II	$CH_2\underline{C}H{=}CH$	130.5
III	$CH{=}\underline{C}H{-}CH_2{-}\underline{C}H{=}CH$	129.1
IV	$CH{=}CH{-}\underline{C}H_2{-}CH{=}CH$	26.7
A, C	CH_2, glycerol	64.4
B	CH, glycerol	72.6
D	OCH_2, serine	65.4
E	NCH, serine	56.1
F	$\underline{C}O_2^-$ serine	172.9

[a] See fig. 1A.

was seen on the interaction of PLP with PS (table 4): a significant change was seen in the relaxation times of the carbon nuclei at the terminal end of the chain and unsaturated carbons but there was little or no change for the nuclei in the methylene chain. Binding of cytochrome c to PS caused no significant change in the relaxation times of any of the nuclei along the chain.

Table 2
Intensity ratios for the ^{13}C resonances of phosphatidylserine with (A_{PL}) and without (A_L) basic protein [a]

Resonance	Assignment	A_{PL}/A_L
1	t-CH_3	1.02 ± 0.03
2	$\underline{C}H_2CH_3$	1.03 ± 0.10
4–15	$(CH_2)_n$	1.07 ± 0.16

[a] The values were averaged for four different samples.

7. Discussion

The observed relaxation times exhibit the trend described previously for several other lipids [4,8,9]: there is an increase in relaxation times away from the glycerol backbone of the phospholipid, which has been attributed to decreased motional restriction toward the bilayer centre. Overall the relaxation times of PS in vesicles are shorter than for other pure or mixed lipid systems studied at the same temperature [2,13], but they follow a relaxation time gradient close to that observed for yeast cells at 26°C [1]. Table 3 also shows that decreasing unsaturation (estimated by comparing the intensities of the unsaturated carbon resonances to those of the methylene envelope) decreases T_1 most for nuclei near the centre of the hydrocarbon chain, with smaller effects near the terminal methyl groups. Presumably this results from the location of most double bonds near C9–C10, with only local effects on chain order and mobility.

Table 3
^{13}C spin–lattice relaxation times (in seconds) for phosphatidylserine vesicles without (T_{1L}) and with (T_{1PL}) basic protein [a]

Assignment	T_{1L}	T_{1PL}	T_{1L}	T_{1PL}	T_{1L}	T_{1PL}	Estimated % error.
t-CH_3	1.82	0.94	1.87	1.59	1.67	1.35	5
$\underline{C}H_2CH_3$	0.62	0.39	0.67	0.55	0.57	0.46	5
$\underline{C}H_2CH_2CH_3$	0.33	0.25	0.34	0.315	0.36	0.30	5
$(CH_2)_n$	0.202	0.183	0.236	0.201	0.291	0.285	5
$\underline{C}H_2CH{=}CH$			0.227	0.164	0.225	0.208	10
$CH_2\underline{C}H{=}CH$			0.30	0.24	0.37	0.31	10
$CH{=}CH{-}\underline{C}H_2{-}CH{=}CH$					0.231	0.208	10
$CH{=}\underline{C}H{-}CH_2{-}\underline{C}H{=}CH$					0.53	0.49	10
$\underline{C}H_2C{=}O$	0.104	0.085	0.162	0.140	0.128	0.114	10
$\underline{C}H_2CH_2C{=}O$	0.118	0.093	0.184	0.163	0.203		10

[a] The times are for three batches of lipid, the degree of unsaturation increasing from the left column to the right column. All samples were in deuterated 5 mM Tris buffer containing 1 mM EDTA at pD 9.5 and 31°C.

Table 4
^{13}C spin-lattice relaxation times (in seconds) for phosphatidylserine vesicles without added protein (T_{1L}) and with added cytochrome c (T_1 cyt c), proteolipid protein (T_{1PLP}) or basic protein (T_{1MBP})

Assignment	T_{1L}	$T_{1cyt\ c.}$	T_{1L}	T_{1PLP}	T_{1L}	T_{1MBP} [a]	Estimated % error.
t-CH_3	1.67	1.74	1.59	1.32	1.67	1.36	5
$\underline{CH_2}CH_3$	0.64	0.69	0.60	0.55	–	–	5
$\underline{CH_2}CH_2CH_3$	0.36	0.34	0.40	0.34	–	–	5
$(CH_2)_n$	0.282	0.284	0.291	0.283	0.291	0.256	5
$\underline{CH_2}CH{=}CH$	0.251	0.278	0.262	0.248	–	–	10
$CH_2\underline{CH}{=}CH$	0.36	0.34	0.34	0.29	–	–	10
$CH{=}\underline{CH}{-}\underline{CH_2}{-}\underline{CH}{=}CH$	0.526	0.471	0.52	0.47	0.53	0.44	10
$CH{=}CH{-}\underline{CH_2}{-}CH{=}CH$	0.34	0.30	–	–	–	–	10
$\underline{CH_2}C{=}O$	0.153	0.175	0.125	0.150	–	–	10
$\underline{CH_2}CH_2C{=}O$	0.203	0.180	–	–	–	–	10

[a] The basic protein was co-sonicated with the lipid prior to measurement of the relaxation times.

Significant reductions in the intensity of the methylene envelope with respect to the terminal methyl resonance are evident on binding cytochrome c to PS vesicles (this work) and mixed cardiolipin: phosphatidylcholine vesicles [13]. Brown and Wüthrich concluded that each protein molecule interacts strongly with the headgroups of a number of lipid molecules, broadening beyond detection the resonances from these molecules. Changes in the relaxation times would then represent only long-range perturbations of bilayer motion consequent on protein inclusion. After carefully measuring the intensities of several PS peaks, before and after the addition of basic protein, we have no evidence for a similar intensity loss with this protein.

It is improbable that this lack of intensity changes results from a fortuitous cancellation of effects caused by a reduction in the number of nuclei contributing to a peak (cf. cytochrome c) and a concomitant increase in their nuclear Overhauser enhancement. Dipole–dipole interactions are considered to provide the only mechanism for spin-lattice relaxation for lipid vesicle carbon nuclei, the maximum nuclear Overhauser enhancement being observed for clearly resolved resonances [9]. Within the experimental errors, each set of relaxation data were also described by a single exponential function. We thus conclude that the observed relaxation times are likely to represent an ensemble average, the ensemble including molecules adjacent to protein molecules and those more remote, exchanging (by lateral diffusion) at a rate sufficient to average the relaxation times for each nucleus.

Few studies have been made of the effects of added molecules on the motion and order of lipid molecules in bilayers. The only peripheral protein examined, cytochrome c, clearly has little effect on relaxation rates for nuclei in the acyl chains. In contrast, several intrinsic membrane proteins, including high density lipoprotein apoprotein [12], cytochrome oxidase [15] and the myelin proteolipid protein, are uniform in reducing the relaxation times. In this respect the basic protein bears quantitative similarity in its effects to the integral membrane proteins. Cholesterol is at present the only molecule that penetrates the hydrophobic region which appears to be without influence on relaxation rates of the nuclei near the bilayer centre [‡] [2,11].

In recent years several attempts have been made to develop models of bilayers consistent with observed spin–lattice and spin–spin relaxation rates, nuclear Overhauser effects, and order parameters. Lee et al. [8], using the approximation of unhindered and independent rotation about C–C bonds, could fit their limited ^{13}C relaxation data using separate correlation

[‡] Addition of cholesterol has, in one study [2], caused small but consistent decreases in the relaxation times of nuclei, in much the same manner as basic protein. However the authors considered these reductions to be within the accuracy of their measurements.

times for C—C bond isomerization and for (slower) reorientation of the acyl chains about their long axes. Using an algorithm based on the model of Levine et al. [33,34] for this calculation we attempted to simulate the partial or complete penetration of the protein into the bilayer by reducing the diffusion rates for the first eight bonds or for all of the C—C bonds in the acyl chain. It was found that the decrease in T_1 was approximately equal for each nucleus and no differential effects for one end of the chain were observed. A parallel effect resulted from reduction of the correlation time for axial molecular motion (from 10^{-9}, to 10^{-8}, to 10^{-7} s). Recently more sophisticated models that introduce contributions to T_1 from variable C—C bond isomerization rates, segmental order, motional correlation along the acyl chain, the cumulative effects of rotational isomerization have been formulated [10]. Explicit expressions of T_1 as a function of both rotational correlation time and order parameter have been derived [32]. But even these equations can provide no non-empirical correction for motional correlation. Thus, although much of the T_1 gradient in pure lipid vesicles is thought to result from local changes in the rate of gauche—trans isomerization, and there is evidence that order makes a minor contribution to ^{2}H relaxation times [32] (which are directly related to ^{13}C relaxation times), ^{13}C relaxation rates alone are not sufficient to provide an unambiguous quantitative description of lipid motion and the effects of perturbants. Some qualitative conclusions can however be drawn.

The observation that basic protein reduces the effective correlation times for nuclei close to the bilayer centre is consistent with its penetration into the hydrophobic region of the bilayer, especially in view of the behaviour of other integral proteins. Such penetration is also indicated by other effects of this protein on monolayer and bilayer structure: it lowers the phase transition temperature and enthalpy [25, 35], increases vesicular permeability [36], expands monolayers [37], binds uniformly to PS and PC vesicles over a wide ionic strength range [19], and alters the order and rate of motion of spin labels intercalated into bilayers [25]. It has been reported that basic protein penetrates other lipids, such as dipalmitoylphosphatidylglycerol and cerebrosides [25,37] more readily than PS. Participation of hydrophobic protein residues in bilayer binding has also been proposed on the basis of ^{1}H NMR studies with detergent micelles [38].

Table 5
Spin—lattice relaxation times (T_1) for various lipids

Lipid	Temperature/°C	Relaxation times/s		Ref.
		ω [a]	$(\omega-1)$ [a]	
didecanoyl-PC	52	3.67 ± 0.09	2.03 ± 0.07	[8]
didodecanoyl-PC	52	3.85 ± 0.06	2.09 ± 0.09	[8]
dimyristoyl-PC	52	3.81 ± 0.08	1.70 ± 0.10	[8]
dipalmitoyl-PC	52	3.34 ± 0.05	1.81 ± 0.08	[8]
eggPC	50	3.70	1.69	[9]
1-decanol	42	3.1	2.2	[39]
octadecane	52	4.71 ± 0.18	3.72 ± 0.15	[34]
(octadecane	31	3.57 ± 0.12	1.59 ± 0.02)	[34]

[a] The terminal methyl group of the acyl chain is designated ω, and the penultimate carbon is designated $(\omega - 1)$.

On the basis of the relaxation data we cannot surely distinguish between partial penetration and a direct interaction of the protein with nuclei near the bilayer centre. But there are several indications that the relaxation rate of the terminal methyl group is unlikely to change unless it is directly motionally restricted. As is shown in table 5, the methyl T_1 is not dependent on the length of the acyl chain in PC vesicles [8] or alkyl bromides [34], and hence is not determined by cumulative motions along the chain. It is unaffected by the presence of double bonds in the chain (as in egg PC, table 5, or PS, table 3) or of cholesterol, although these alter the order and motion in most of the acyl chain. Thirdly, the effective correlation times for bilayer systems and for molecules such as octadecane and (particularly) decanol are not appreciably different, despite considerable differences for their order parameters and the relaxation rates of nuclei removed from the chain terminii.

It is apparent from this and previous work that if a protein interacts hydrophobically with the lipid bilayer then a decrease in the relaxation times of nuclei at the terminal region of the hydrocarbon chain will result, but a similar change nearer the headgroup need not occur. For extrinsic proteins, such as cytochrome c (under some conditions), where minimal disruption of the bilayer structure is to be expected, there will be little or no alteration in the longitudinal relaxation times.

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