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Transverse nuclear spin relaxation in phosphatidylcholine bilayers containing gramicidin

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Deuterium nuclear magnetic resonance has been used to study transverse relaxation in samples of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, perdeuterated and specifically deuterated at the α position of the chains, containing the polypeptide gramicidin at concentrations of 0, 1, and 4 mol%. For 4 mol% gramicidin, the bilayer is thought to undergo a continuous phase change rather than a phase transition proceeding via two phase coexistence. Information is obtained regarding lipid dynamics in the continuous phase change region of the phase diagram. In the presence of gramicidin, the transverse relaxation time measured by the quadrupole echo technique, T_{2e}, passes through a minimum in the gel phase. The gramicidin concentration dependence of T_{2e} suggests that the polypeptide reduces the temperature sensitivity of the correlation time responsible for the minimum. The polypeptide also increases the sensitivity of the first spectral moment, M_1, to the quadrupole echo pulse separation. This behavior is attributed to a polypeptide-induced enhancement of the spread in T_{2e} along the acyl chains. Quadrupole Carr-Purcell-Meiboom-Gill experiments are used to separate contributions to the observed behavior from fast and slow motions.

Introduction

Deuterium nuclear magnetic resonance (^{2}H-NMR) has been used extensively to study lipid–protein and lipid–polypeptide interaction. Recent reviews have dealt with applications of ^{2}H-NMR to studies of protein-induced changes in lipid orientational order [1,2] and dynamics [3]. It has also been possible to use calorimetry and spectroscopy in a complementary manner to determine polypeptide-lipid phase diagrams [4].

Bloom and Smith [2] have discussed protein-induced effects found using ^{2}H-NMR to observe labeled lipids in the bilayer. Protein has little effect on the spin lattice relaxation time, T_1, implying little change in motions with correlation times of order 10^{-9}s to 10^{-8}s. In the liquid crystalline phase, protein also has little effect on average orientational order. Protein does, however, influence transverse relaxation. The quadrupole echo decay time, T_{2e}, in the liquid crystalline phase is significantly reduced by protein implying an effect on motions with correlation times longer than 10^{-7} s. This effect has been observed for reconstitutions containing a variety of proteins including rhodopsin [5,6], cytochrome-*c* oxidase [7,8], and myelin proteolipid apolipoprotein [9]. One of the most profound effects of the lipid–protein interaction is the modification of bilayer phase behavior.

Gramicidin is a naturally occurring antibiotic polypeptide that is known to form bilayer spanning dimer channels [10–12]. Depending on the sample preparation, it is also possible to obtain non-channel forms of gramicidin in bilayers [13–15]. Gramicidin has often been used as a model for lipid–protein interaction studies as summarized elsewhere [16]. It has recently been suggested that the phase diagram for gramicidin in PC contains a small teardrop shaped region of two phase coexistence. For gramicidin concentrations beyond about 2 mol%, the first order gel-liquid crystal transition is replaced by a continuous phase change [16,17]. In passing continuously from a liquid crystalline state to a more gel-like state, the lipid presumably explores dynamic and conformational conditions which are bypassed when the change occurs abruptly in a phase transition.

The present work was undertaken to explore the sensitivity of lipid dynamics to polypeptide concentration in the vicinity of the critical mixing point. T_{2e} and T_1 have been measured for 4 mol% and 1 mol% mixtures of gramicidin D in perdeuterated dimyristoylphosphatidylcholine (DMPC-d_{54}) bilayers and 4 mol%

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gramicidin in α-deuterated DMPC bilayers (DMPC-d_4). These have been compared to results using pure DMPC-d_{54} and DMPC-d_4 bilayers. Quadrupole Carr-Purcell-Meiboom-Gill (q-CPMG) experiments [18] have been used to determine the relative contributions of fast and slow motions to the transverse relaxation rate in the continuous phase change region of the phase diagram.

Materials and Methods

DMPC-d_{54} was synthesized by the method of Gupta et al. [19] with fatty acid perdeuterated according to the method of Hsiao et al. [20]. DMPC-d_4 was synthesized in the same way with fatty acid deuterated using the method of Nguyen and Stenhagen [21]. Gramicidin D (Dubos) was purchased from Sigma (Sigma-Aldrich Corp., St. Louis, MO, U.S.A.). In preparing the sample concentrations, gramicidin was assumed to have a molecular weight of 2000. The molecular weight of DMPC-d_{54} was taken to be 767 assuming two waters of hydration. The same assumption was applied for DMPC-d_4 so that the molecular weight was taken to be 717. Stock solutions of lipid and gramicidin were prepared in chloroform/methanol (2 : 1, v/v). Samples were prepared by mixing appropriate volumes of stock solution and then drying them to a thin film using rotary evaporation followed by overnight pumping at room temperature. Samples of DMPC-d_{54} were prepared with 0, 1, and 4 mol% gramicidin. Samples of DMPC-d_4 were prepared using 0, and 4 mol% gramicidin. Samples with dry weights of from 40 mg to 70 mg were placed into 8 mm NMR tubes and hydrated with 200 to 300 μl of 50 mM phosphate buffer at a pH of 7.0. The sample of 4 mol% gramicidin in DMPC-d_4 had a dry weight of 260 mg. Samples were stirred in the NMR tube with a fine glass rod and found to run as a single spot when checked with thin-layer chromatography.

^{2}H-NMR experiments were performed at 23 MHz using a superconductive solenoid and a 'home-built' spectrometer. A Nicolet 2090 oscilloscope was used to digitize transients which were averaged in a Tandy 1200 microcomputer. A second microcomputer controlled the temperature of a copper oven surrounding the sample tube. Samples were allowed to equilibrate for at least half an hour at each temperature before data collection was begun. Spectra were collected using a phase cycled quadrupole echo pulse sequence. To begin the Fourier transform from the top of the echo, it was generally necessary to shift the points in the accumulated signal by some fraction of a dwell time [1]. To improve the signal to noise ratio, even and odd points in the accumulated signal were shifted separately to give two free induction decays with points at the echo maximum. These were added to yield a contracted free induction decay with an effective dwell time twice that of the original signal. For samples containing DMPC-d_{54}, signals were collected with dwell times of 1 μs in the gel phase and 2 μs in the liquid crystal phase and contracted to give effective dwell times of 2 μs and 4 μs, respectively. For samples containing DMPC-d_4, transients were collected with dwell times half the size of those for the perdeuterated lipid samples and the contraction carried out twice to arrive at the same effective dwell times for gel and liquid crystal. The $\pi/2$ pulse length employed was normally between 2.4 and 3.2 μs. For samples containing DMPC-d_{54}, spectra were obtained by averaging up to 4000 transients. For samples containing α-deuterated DMPC-d_4, the number of transients collected ranged from 8000 to 64000 depending on T_{2e} and the size of the sample.

Spectra were collected for each sample using the quadrupole echo sequence [22] with a pulse spacing of 35 μs in order to determine first spectral moment (M_1) as a function of temperature in the region of the gel-to-liquid-crystal transition. In addition, quadrupole echo experiments with different pulse separations were performed in order to determine the quadrupole echo decay time, T_{2e}. For exponential decay, the signal intensity is given by

$$A(2\tau) = A(0)e^{-2\tau/T_{2e}} \tag{1}$$

For perdeuterated samples, variation of T_{2e} along the chain results in non-exponential decays. Unless otherwise indicated, the values of T_{2e} reported for samples containing DMPC-d_{54} were determined from the initial decay of the echo and thus represent an average over all chain positions and molecule orientations. The dependence of M_1 on τ was also determined from these experiments. Inversion recovery experiments were performed on the samples containing DMPC-d_{54} at temperatures bracketing the transition in order to determine the sensitivity of T_1 to protein concentration.

Finally, a modified q-CPMG experiment (Ref. 18) $(90_x\text{-}\tau\text{-}(90_y\text{-}2\tau\text{-})N)$ was performed on the 4 mol% gramicidin in DMPC-d_{54} sample in order to explore relative contributions of fast and slow motions to transverse relaxation. If there exist motions with correlation times long (τ_{cl}) and short (τ_{cs}) compared to τ, then the apparent transverse relaxation time can be written as

$$\frac{1}{T_2^{q\text{-}CPMG}} \cong \frac{\Delta M_{2l}\tau^2}{3\tau_{cl}} + \sum \Delta M_{2s}\tau_{cs} \tag{2}$$

where ΔM_{2l} and ΔM_{2s} are those parts of the second moment modulated by the motions with long and short correlation times, respectively. By averaging over position along the chain and over orientation, these expressions can be applied to powder samples containing perdeuterated acyl chain lipids.

In the limit where the separation between echoes in the q-CPMG sequence goes to zero, the contribution to

transverse relaxation from slow motions is removed and the observed echo decay gives the relaxation rate due to the faster motions. Because the pulse programmer was not capable of controlling the digital oscilloscope timebase externally, it was necessary to collect the echo for each value of $2n\tau$ separately.

Results

Fig. 1 shows the temperature dependence of the spectrum for DMPC-d_{54} with 4 mol% gramicidin. The change from liquid crystal to gel type spectra proceeds via a continuous evolution of spectral characteristics with decreasing temperature. This is consistent with the gramicidin concentration being beyond a teardrop-shaped two phase region in the binary phase diagram [16].

Fig. 2 shows the temperature dependence of the first spectral moment (M_1) for three samples containing DMPC-d_{54}. The continuous nature of the phase change in the 4 mol% gramicidin sample, in contrast to the discontinuous transition observed in the pure lipid sample, is evident. The 1 mol% gramicidin sample displays some evidence of a continuous phase change but this is interrupted by the transition. The dependence of M_1 on gramicidin concentration suggests that gramicidin partially orders the bilayer above the transition. As discussed below, some care must be taken in interpreting M_1 below the pure lipid transition temperature.

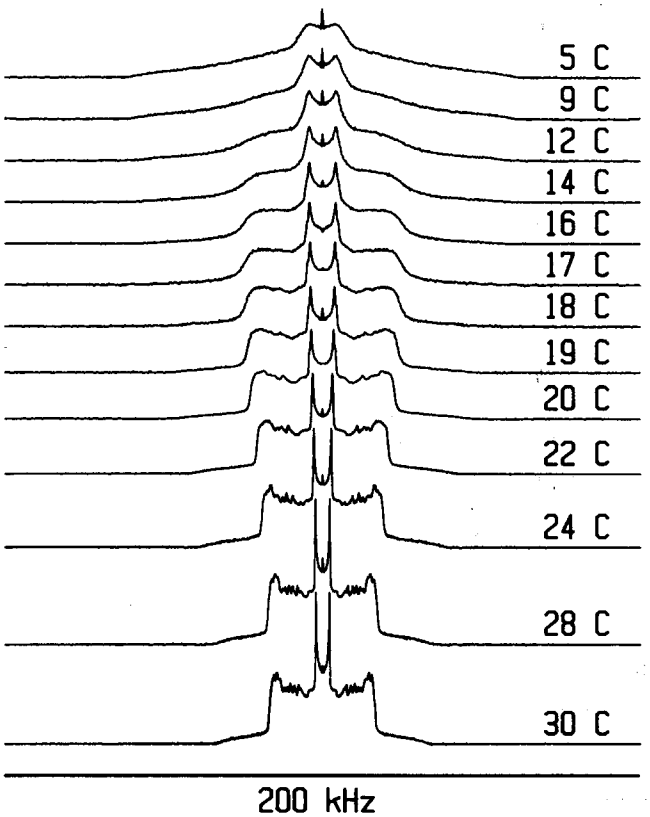

Fig. 1. Temperature dependence of the ^{2}H-NMR spectrum for DMPC-d_{54} containing 4 mol% gramicidin.

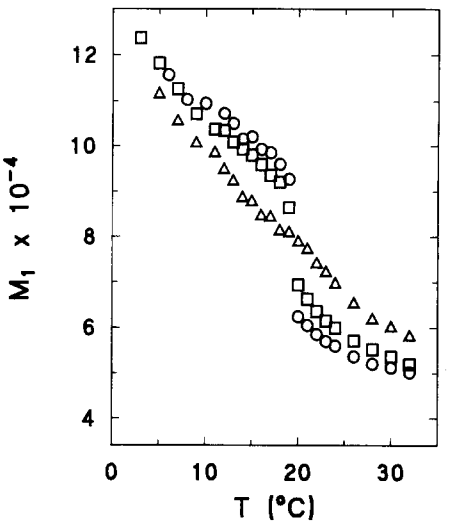

Fig. 2. Temperature dependence of the first spectral moment for DMPC-d_{54} (○), DMPC-d_{54} with 1 mol% gramicidin (□) and DMPC-d_{54} with 4 mol% gramicidin (△).

Inversion recovery experiments on DMPC-d_{54} samples containing 1, and 4 mol% gramicidin at 23°C and 16°C (not shown) indicate that the presence of gramicidin does not influence T_1 in either phase. This shows that gramicidin is not influencing motions with correlation times between about 10^{-8}s and 10^{-9}s.

Quadrupole echo spectra were collected, using a series of pulse separations, for DMPC-d_{54} samples containing 0, 1, and 4 mol% gramicidin. These spectra were analyzed to obtain T_{2e} averaged over the chain and, below the transition, T_{2e} for the methyl deuterons. The dependence of M_1 on τ, for these spectra, provided an indication of the variation in T_{2e} with position along the chain.

Fig. 3 shows the dependence of the area under the spectrum, M_0, on the quadrupole echo delay, 2τ, at a number of temperatures for the sample containing 4 mol% gramicidin in DMPC-d_{54}. Non-exponential decay seen at 17°C is the consequence of there being a range of transverse relaxation rates for different sites along the chain. In particular, the narrowest spectral component, due to the methyl groups, decays more slowly than the rest of the spectrum. As discussed below, the variation in T_{2e} along the chain gives rise to a strong τ dependence of M_1. This effect is most evident just below the transition.

The values of T_{2e} for the DMPC-d_{54} samples are shown by open symbols in Fig. 4. These are obtained by least-squares fits to the initial decay in data of the type shown in Fig. 3. For decays in which no departure from exponential behavior was observed, the fit was performed using the complete data set. For the more non-exponential decays, the data set was restricted to

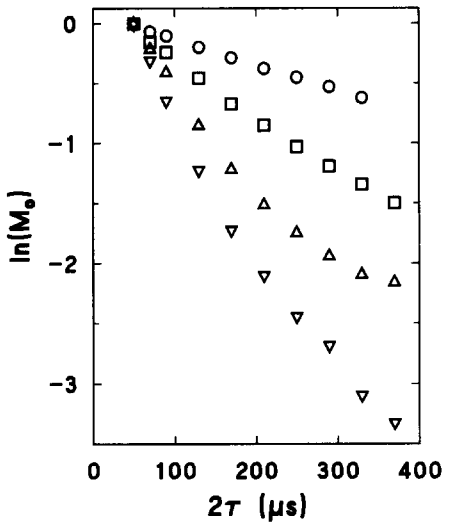

Fig. 3 In M_0 versus 2τ for the quadrupole echo experiment on DMPC-d_{54} containing 4 mol% gramicidin. ○, 24°C; □, 19°C; △, 17°C; ▽, 9°C.

$2\tau < 200$ μs. At the liquid-crystal-to-gel transition of the pure lipid sample, T_{2e} abruptly decreases by a factor of about 8. Below the transition, T_{2e} increases slowly with decreasing temperature. The discontinuity in T_{2e} is similar to that reported by Meier et al. [23]. The sample with 1 mol% gramicidin undergoes a less abrupt change in T_{2e} in the neighborhood of the transition and displays a distinct minimum. The lowest value of T_{2e}, 92 μs, occurs near 17°C. With 4 mol% gramicidin, T_{2e} is lowered slightly in the liquid crystalline phase and undergoes an even more gradual decrease in the neighborhood of 20°C. The minimum is not as well defined but appears to be about 70 μs near 9°C.

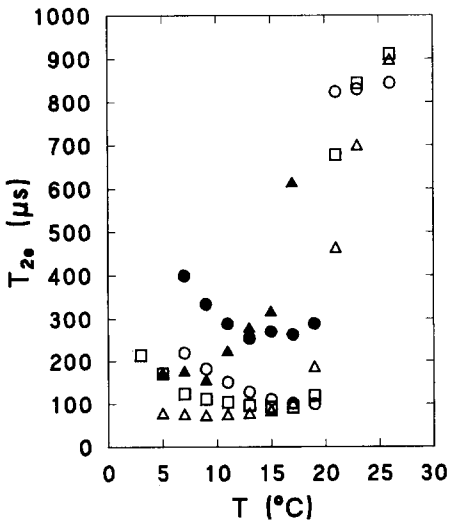

Fig. 4. T_{2e} versus temperature for DMPC-d_{54}, (○), DMPC-d_{54} with 1 mol% gramicidin (□) and DMPC-d_{54} with 4 mol% gramicidin (△). The closed symbols indicate methyl group relaxation for DMPC-d_{54} (●) and DMPC-d_{54} with 4 mol% gramicidin (▲).

The decay of the methyl component for the perdeuterated lipid samples in the gel phase was determined using spectra obtained with τ greater than 85 μs. For these spectra, the methylene contributions to the spectrum have decayed sufficiently to allow the limits of the methyl spectrum to be reasonably approximated. By subtracting the remaining methylene component, it was possible to estimate T_{2e} for the methyl groups in the gel phase. These are shown, for DMPC-d_{54} and for the DMPC-d_{54} sample with 4 mol% gramicidin, as solid symbols in Fig. 4.

The data in Fig. 4 suggest that, just below the transition, gramicidin influences the T_{2e} for the methyl deuterons significantly more than for the rest of the chain. A particularly sensitive indication of a spread in T_{2e} along the chain, for perdeuterated lipid, is the dependence of M_1 on 2τ. Defining $f_i(\omega)$ to be that part of the spectrum associated with a particular deuteron site, the intensity associated with that spectral component is

$$M_{0i} = \int f_i(\omega)\, d\omega \tag{3}$$

and the first moment of that spectral component is

$$M_{1i} = \frac{\int f_i(\omega)\,\omega\, d\omega}{M_{0i}} \tag{4}$$

If the ith spectral component has a specific quadrupole echo decay time, T_{2ei}, then it can be shown that

$$\lim_{\tau \to 0} \frac{d \ln M_1}{d2\tau} = -\frac{1}{M_0 M_1(0)} \sum_i \frac{M_{0i}}{T_{2ei}} (M_{1i} - M_1(0)) \tag{5}$$

where $M_0 = \Sigma_i M_{0i}$ and $M_1(0)$ is the first spectral moment for $\tau = 0$. M_1 will display a τ dependence if there are components of the spectrum with M_{1i} not equal to $M_1(0)$ and if T_{2ei} is not a constant for all components.

Fig. 5 shows the initial slopes of ln M_1 versus 2τ as a function of temperature for the three samples containing DMPC-d_{54}. For temperatures with more exponential dependencies of M_1 on 2τ, the initial slopes have been calculated by fitting the data with $2\tau < 200$ μs. For temperatures where the dependence of M_1 on 2τ is most non-exponential, the initial slope was determined from the first 90 μs.

The data in Fig. 5 indicate that, near and below the transition, gramicidin enhances the spread in T_{2e} along the chain. The resulting dependence of M_1 on 2τ distorts the picture of the phase transition presented by M_1 versus T in Fig. 2. Accordingly, M_1 was extrapolated back to $\tau = 0$ for those temperatures and polypeptide concentrations for which T_{2e} data were collected. The results are shown in Fig. 6. While an extrapolation based on the type of data in Fig. 5 is only approximate, it is clear that the corrected M_1 values for the 4 mol%

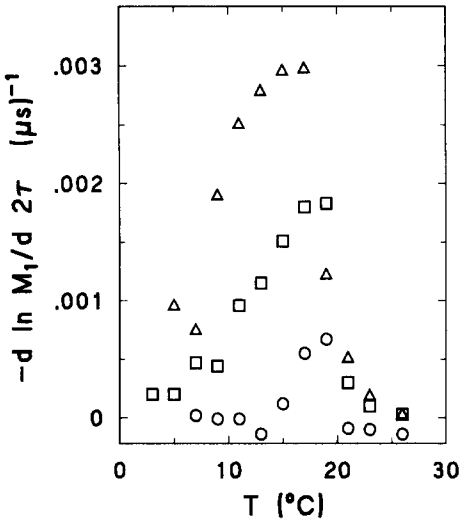

Fig. 5. $-\mathrm{d}\ \ln M_1/\mathrm{d}2\tau$ versus temperature for DMPC-d_{54} (○), DMPC-d_{54} with 1 mol% gramicidin (□) and DMPC-d_{54} with 4 mol% gramicidin (△).

gramicidin sample indicate that rather than disordering the lipid bilayer below 19 °C, the polypeptide has little effect on M_1 and thus on order in the bilayer gel phase. This presents a rather different picture of the continuous phase change and suggests that care must be taken in using perdeuterated sample moments to investigate phase transitions in the presence of other molecules such as polypeptides.

The tendency of gramicidin to partially order the lipid above the transition is consistent with results from Raman [24] and infrared [25] studies of this system. Such studies [25,26] also indicate that gramicidin disorders the lipid below the transition. The corrected values of M_1 below the transition in Fig. 6, however, show little disordering.

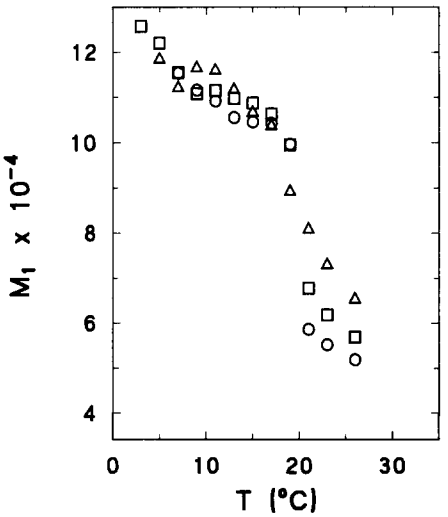

Fig. 6. M_1 extrapolated to $\tau = 0$ versus temperature for DMPC-d_{54} (○), DMPC-d_{54} with 1 mol% gramicidin (□) and DMPC-d_{54} with 4 mol% gramicidin (△).

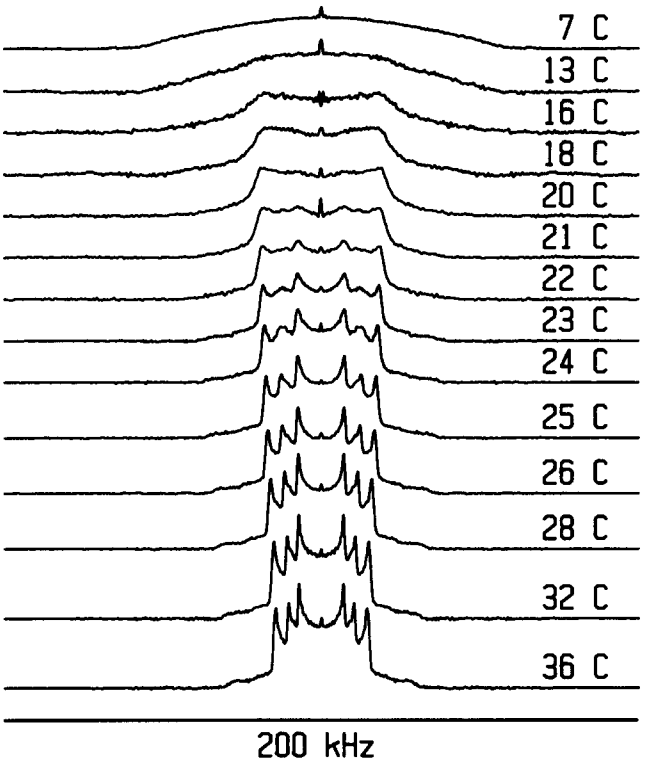

Fig. 7. Temperature dependence of the ^{2}H-NMR lineshape for α-deuterated DMPC containing 4 mol% gramicidin.

The results given above suggest that the polypeptide amplifies the range of T_{2e} values experienced by different deuterons along the chains. To determine the range of T_{2e} along the chain more directly, quadrupole echo experiments were also performed on samples containing DMPC-d_4. The dependence of the lineshape on temperature for DMPC-d_4 containing 4 mol% gramicidin, using a quadrupole echo pulse separation of 35 μs, is shown in Fig. 7. The transition temperature for the α-deuterated lipid shows essentially no isotope effect so that the pure lipid transition is close to the normal transition temperature of 23.9 °C [27]. The absence of a superposition of gel and liquid crystal spectra in the neighborhood of the pure lipid transition temperature confirms the continuous nature of the phase change in this region of the phase diagram. The spectra show interesting changes between 25 °C and 22 °C. The three splittings observable are identified with three distinct α-deuteron environments in the liquid crystalline phase [28]. The two narrowest splittings are from the inequivalent deuterons on the chain at position 2. The largest splitting comes from deuterons on the chain at position 1. It is interesting that the spectral component associated with one of the inequivalent deuterons begins to lose its axially symmetric nature while the geminal deuteron does not. This might provide an interesting problem for spectral modeling [23,29].

The limited effect of gramicidin on bilayer order below the transition, suggested by Fig. 6, is partially

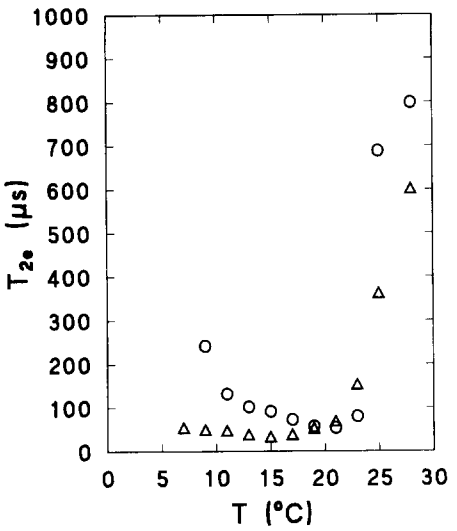

Fig. 8. T_{2e} versus temperature for α-deuterated DMPC (○) and α-deuterated DMPC containing 4 mol% gramicidin (△).

confirmed by M_1 measurements on DMPC-d_4 containing 4 mol% gramicidin (not shown) which suggest that gramicidin-induced disordering of the bilayer below 15 °C is small. The spectrum for the specifically labeled sample is not distorted by the variation in T_{2e} along the chain. It does not, however, reflect the average behavior along the chain. The smaller signal to noise ratio for the specifically labeled sample also increases the error in M_1 measurements because of the weighting of wing intensity in the moment calculation.

Fig. 8 shows T_{2e} versus T for DMPC-d_4 and for 4 mol% gramicidin in DMPC-d_4. For the sample containing gramicidin, the T_{2e} minimum is about 29 μs at 15 °C. Given that the T_{2e} minimum observed for the perdeuterated sample is the minimum in the value of T_{2e} obtained by averaging the relaxation rates rather than the average value of the T_{2e} minima, it is not surprising that the specifically deuterated sample displays a minimum that is lower than for the perdeuterated sample. That the minimum appears to occur at a slightly higher temperature is presumably a consequence of the difference in isotope effect for the perdeuterated and α-deuterated samples. For DMPC-d_4 containing 4 mol% gramicidin, M_1 is not found to depend on τ. This indicates that the τ depenence of M_1 in the perdeuterated samples reflects the effect of gramicidin on the distribution of transverse relaxation times along the chain rather than a gramicidin-induced change in the orientational dependence of transverse relaxation.

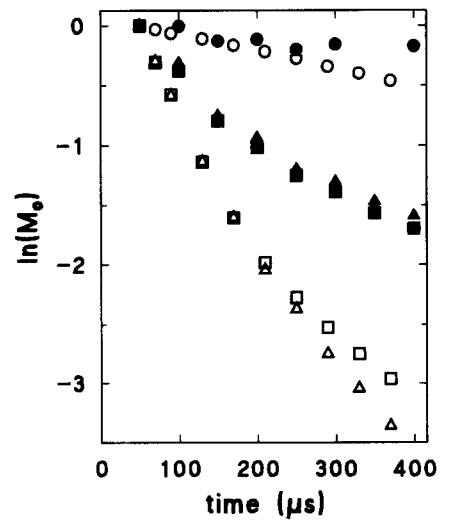

Fig. 9. Echo decay for DMPC-d_{54} containing 4 mol% gramicidin. Open symbols show ln M_0 versus 2τ for quadrupole echo experiments at 23 °C (○), 13 °C (□), and 6 °C (△). Closed symbols show ln M_0 versus $2n\tau$ for q-CPMG experiments with $\tau = 25$ μs at 23 °C (●), 13 °C (■), and 6 ° (▲).

In order to explore the relative importance, across the continuous phase change, of transverse relaxation contributions from fast and slow motions, quadrupole CPMG experiments [18] were performed on the 4 mol% gramicidin in DMPC-d_{54} sample at 23 °C, 15 °C, 13 °C, and 6 °C.

Fig. 9 shows ln M_0 (normalized to the first echo) versus 2τ for the normal quadrupole echo experiment and versus $2n\tau$ for the quadrupole CPMG experiment with $\tau = 25$ μs. A τ of 25 μs is sufficiently close to zero that the CPMG experiment should effectively refocus the decay due to slow motion. The slope of the q-CPMG line thus gives an upper limit for the transverse relaxation rate due to fast motions while the difference between the quadrupole echo and q-CPMG slopes places a lower limit on the relaxation rate due to slow motions. For 15 °C, 13 °C and 6 °C, the contribution to T_{2e}^{-1} due to fast motions is less than 7 500 s^{-1} while the contribution due to the slow motion is greater than 5 500 s^{-1}. Bloom and Sternin [18], using a q-CPMG experiment, found that for DPPC-d_{31} at 44 °C, the contribution to T_{2e}^{-1} due to fast motions was 625 s^{-1}. Using their quadrupole echo relaxation rate, it is possible to deduce that the slow motion contribution was about 1 400 s^{-1}. This motion was identified as diffusion around the curved vesicle surface. It is presumably the relative importance of the slow motion contribution to transverse relaxation that renders T_{2e} measurements in the liquid crystalline phase so sensitive to vesicle size.

Discussion

Before discussing the NMR results presented here, it is useful to consider some points regarding the conformation of gramicidin. Circular dichroism measurements (Taylor, L. and Simatos, G., personal communication) on the 4 mol% gramicidin in DMPC-d_{54} samples following the NMR experiments reported here indicate a mixture of channel and non-channel gramicidin conformation based on comparison with the results of LoGrasso et al. [15]. The authors of reference [15] use

solid-state NMR experiments on uniformly ^{15}N-labeled gramicidin A in oriented bilayers and DSC results to suggest that the non-channel form remains in intimate contact with the fatty acyl lipid chains and that its orientation is similar to that of the channel conformation. NMR experiments done in this laboratory on samples containing gramicidin in the channel conformation indicate that the effects of channel and non-channel gramicidin on the surrounding lipid are qualitatively similar (Morrow, M., Taylor, L., Simatos, G. and Srinivasan, R., unpublished results) suggesting that the degree of contact between the polypeptide and fatty acyl chains is similar. The non-channel conformation thus remains an interesting model for a bilayer-spanning component as long as the systems are prepared in a consistent manner. It should be noted that one NMR experiment using [^{15}N-Val_7]-gramicidin A in randomly oriented dispersions implied that near the Val_7 site, the channel and non-channel conformations of gramicidin display very different backbone structure and dynamics [14].

The results presented here can be discussed in terms of the effect of gramicidin on correlation times below the transition as indicated by the T_{2e} minima, the effect of gramicidin on the distribution of T_{2e} along the chain, and the relative importance of fast and slow motions for transverse relaxation below the transition as indicated by q-CPMG.

The occurrence of a minimum in T_{2e} has been discussed by Pauls et al. [30]. For motions with a correlation time, τ_c, which modulate a portion ΔM_2 of the second moment, one can identify a fast motion regime where $\Delta M_2 \tau_c^2 \ll 1$ and a slow motion regime where $\Delta M_2 \tau_c^2 \gg 1$. In the fast motion regime, $1/T_{2e} = \Delta M_2 \tau_c$ [30,31]. In the slow motion regime, T_{2e} increases with increasing τ_c as long as the pulse separation, τ, satisfies $\tau \gg \Delta M_2^{-1/2}$. The minimum in T_{2e} between these regimes can be interpreted in terms of various motional models. In practice, the analysis is limited by the difficulty of determining the appropriate ΔM_2 in the presence of more than one motion.

The observed relaxation rate for the perdeuterated samples is a weighted average along the chain and the T_{2e} minimum observed is not the average of the T_{2e} minima but rather the minimum in the value of T_{2e} obtained by averaging the relaxation rates. In the gel phase, gramicidin changes the temperature dependence of T_{2e} for a given site along the chain. In general, increasing the gramicidin concentration reduces the value of T_{2e} at the minimum, moves the minimum to lower temperature, and broadens the minimum. Figs. 4 and 8 indicate that this effect is seen both at the α position and for the methyl groups as well as in the chain averaged value of T_{2e}. Qualitatively, then, the temperature dependence of T_{2e} for all positions along the chain appears to be altered in a similar way. The observation that gramicidin has little effect on the chain averaged T_{2e} immediately below the transition and that it significantly reduces T_{2e} for lower temperatures suggests that the polypeptide influences transverse relaxation by reducing the temperature sensitivity of the correlation time for that part of the motion which is responsible for the presence of the minimum. The indication, from Fig. 6, that gramicidin has a limited effect on lipid order in the gel phase supports the contention that the polypeptide acts on the transverse relaxation rate through a correlation time rather than by altering ΔM_2.

While gramicidin alters the shape of the T_{2e} minimum in a qualitatively similar way for all positions along the chain, the magnitude of the effect varies along the chain. The result is that gramicidin increases the range of T_{2e} along the chain in the gel phase particularly between 15°C and 19°C. Below 13°C, the presence of gramicidin appears to reduce T_{2e} for all positions including the methyl and the α deuterons. Above 13°C, however, T_{2e} for the methyl deuterons is increased in the presence of gramicidin while T_{2e} for the α deuterons, and presumably most of the rest, is reduced by the gramicidin. At 15°C for the 4 mol% gramicidin sample, T_{2e} for the α position is about 30 μs while, for the methyl position, it is about 300 μs. In the pure lipid sample, the methyl group T_{2e} is longer than the chain averaged value but displays the same type of temperature dependence. For the methyl deuterons, gramicidin effectively spreads the T_{2e} change due to the transition over a much wider temperature range than for the other deuterons on the chain. While the effect of gramicidin on the methyl deuterons can account for most of the enhanced sensitivity of M_1 to τ, it is not possible to rule out smaller differential effects at other sites along the chain.

The T_{2e} measurements thus indicate that gramicidin influences the temperature dependence of τ_c for whatever motion gives rise to the T_{2e} minimum in the gel phase. The q-CPMG experiments were undertaken in the hope of further characterizing such a motion by investigating the relative importance, to transverse relaxation, of motions which are fast or slow on the q-CPMG time scale. The insensitivity of T_{2e} to gramicidin content just below the transition suggests that while gramicidin influences the temperature dependence of correlation times in the gel phase, it does not dramatically alter the types of motion present. In particular, the relative contributions of fast and slow motions to transverse relaxation in the gel phase may not be very sensitive to small gramicidin concentrations. Accordingly, some general points can be made from comparison of the current q-CPMG results with those from reference [18]. In general, cooling should increase τ_c for all motions. For motions on the short τ_c side of a T_{2e} minimum, the contribution to transverse relaxation

should increase. For motions on the long τ_c side of a T_{2e} minimum, the contribution should decrease. This is consistent with the observation that the relaxation rate contribution from fast motions at 15 °C, in the current sample, is considerably larger than for DPPC-d_{31} at 44 °C if the contributing motions correspond to roughly the same ΔM_2. On the other hand, the contribution to transverse relaxation from motions that are slow on the q-CPMG time scale is also higher in the current experiment than for DPPC-d_{31} at 44 °C. In the liquid crystalline phase, the slow motion was identified with diffusion of the lipid around the curved vesicle surface. Below the transition, one would expect the contribution to transverse relaxation from diffusion to disappear as the correlation time for that motion becomes very long. The results presented here suggest either that other slow motions become important below the transition or that motions which are fast on the q-CPMG time scale in the liquid crystalline phase are slow on that time scale in the gel phase.

Motions which are on the long τ_c side of a T_{2e} minimum in the liquid crystal phase presumably remain so in the gel phase. The observed T_{2e} minimum is thus due to changes in the correlation times for motions which are on the short τ_c side of a minimum in the liquid crystal phase. Presumably, it is through these motions that gramicidin influences the shape of the T_{2e} minimum in the gel phase. The actual value of T_{2e} at the minimum contains contributions both from motions passing through a minimum and from slower motions in which the change in relaxation rate contribution with temperature is monotonically increasing as temperature is lowered. A more detailed analysis will require a systematic q-CPMG study in which the contributions to relaxation from fast and slow motions can be separated over a range of temperatures spanning the minimum.

Despite the problem of separating contributions from various motions, it is clear that transverse relaxation studies in the gel phase show some promise. The utility of T_{2e} in the liquid crystalline phase is compromised by its sensitivity to vesicle size. The current observations suggest that transverse relaxation may be a more reliable indicator of protein–lipid interaction in and below the range of continuous phase change. The q-CPMG results presented here suggest, for the gel phase, that fast motions, on the q-CPMG timescale, influence the transverse relaxation rate at least as much as slower motions. Whatever the nature of slow motions contributing to transverse relaxation in the gel phase, they do not dominate transverse relaxation to the same extent as slow motions in the liquid crystal phase. It would thus appear that transverse relaxation measurements in the gel phase are more likely to measure intrinsic properties of the bilayer than do similar measurements in the liquid crystalline phase.

In summary, gramicidin appears to alter the temperature dependence of motions, near and below the transition, which are responsible for the occurrence of the T_{2e} minimum. This effect is more pronounced for the methyl deuterons than for the average along the chain or the α position. This results in a gramicidin-induced enhancement of the spread in T_{2e} along the chain in the temperature range just below the transition. Within this temperature range, the relative contributions to transverse relaxation from faster motions, which presumably control the shape of the T_{2e} minimum, and from slower motions are similar. While this precludes detailed analysis of the T_{2e} minimum at this time, it also suggests that the sensitivity of transverse relaxation to slow diffusive motions and thus to poorly controlled factors such as vesicle size is reduced in the gel phase for multilamellar vesicles.

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