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High Pressure ^{2}H-NMR Study of the Order and Dynamics of Selectively Deuterated Dipalmitoyl Phosphatidylcholine in Multilamellar Aqueous Dispersions

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ABSTRACT High pressure ^{2}H multipulse NMR techniques were used to investigate the effects of pressure on the structure and dynamics of selectively deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) multilamellar aqueous dispersions. The samples were deuterated on both chains at positions 2, 9, or 13. The deuterium lineshapes, the spin-lattice relaxation times, T_1, and the spin-spin relaxation times, T_2, were measured as a function of pressure from 1 bar to 5 kbar at 50°C for the three deuterated DPPC samples. This pressure range permitted us to explore the phase behavior of DPPC from the liquid-crystalline (LC) phase through various gel phases such as the Gel I ($P_{\beta'}$), Gel II ($L_{\beta'}$), Gel III, Gel X, and the interdigitated, Gel i, gel phase. Pressure had an ordering effect on all chain segments both in the LC phase and various high pressure gel phases as indicated by the increase in S_{CD} bond order parameter and the first moment, $M1$, with pressure. Compared with the adjacent gel phases, the Gel i phase had the highest order. Also, in all gel phases the carbon-9 segment of the chains had the most restricted motions in contrast to the LC phase, where the carbon-2 segment was the most restricted. In the LC phase, T_1 and T_2 values for all segments decreased with pressure, indicative of the fast correlation time regime. Similarly, T_1 decreased with pressure in the Gel I and the interdigitated Gel i gel phases but changed to the slow correlation time regime at the Gel i/Gel II phase transition. For T_2, which reflects slow motions, the transition to the slow correlation time regime occurred already at LC/Gel I phase transition. Considering the various motions which contribute to relaxation, the behavior of T_1 and T_2 in the Gel II through Gel X phases showing discontinuities and slope changes at the phase transitions was, as expected, quite complex. In addition we found a straight line relationship for T_1^{-1} vs. S_{CD}^2, and T_2^{-1} vs. S_{CD}^2 for the deuterons in the 9 and 13 positions in the LC phase in the pressure range investigated.

INTRODUCTION

This study represents a continuation of our systematic NMR investigations (Jonas and Jonas, 1994) of pressure effects on model phospholipid membranes. Of particular relevance to the present study are our recent high pressure deuterium and phosphorous NMR studies of DPPC. The first study (Driscoll et al., 1991) concentrated on the pressure effects on the gel states of DPPC-d_{62} and used high pressure ^{2}H-NMR techniques to detect various gel phases and to generate a pressure-temperature phase diagram for this phospholipid. Gel state spectra were observed at several temperatures and pressures up to 5 kbar. The gel state made up of several phases, each with a particular structure. At ambient pressure, the most often studied are the $P_{\beta'}$ (pretransition) and $L_{\beta'}$ gel phases (Cevc and Marsh, 1987). Also present is a subtransition (L_c) phase that appears upon prolonged refrigeration of the $L_{\beta'}$ phase. No attempt will be made to review here the well known structure and properties of these phases. In contrast to the above well studied thermotropic phases, less is known about the existence and structure of other high pressure/low temperature phases. In this area, Wong et al. (1988) have carried out extensive work using vibrational spectroscopy and have reported the existence of at least five induced by pressure gel phases that they named GI through GV, corresponding to the increasing transition pressures. GI and GII refer to $P_{\beta'}$ and $L_{\beta'}$, respectively, but the structure and dynamics of the GIII, GIV, and GV gel phases are not as well characterized. In addition to Wong's studies, small angle neutron diffraction (Braganza and Worcester, 1986; Winter and Pilgrim, 1989; Winter et. al., 1991) studies have shown the existence of a pressure-induced, interdigitated phase, G_i, in DPPC bilayers at pressures above 1 kbar and temperatures above 40°C. This phase was also detected by Prasad et al. (1987) using light-scattering techniques at high pressure.

Another series of experiments (Driscoll et al., 1991) was performed in our laboratory on pure lipid bilayers of perdeuterated DPPC-d_{62} in the liquid-crystalline (LC) state and on bilayers in the LC state containing the charged form of the local anesthetic tetracaine (TTC). The results showed that the order parameters of all segments of the acyl chains increased with pressure in the LC state. The more highly ordered regions of the chain were affected slightly more than the regions near the methyl ends. The addition of tetracaine increased the disorder of the chains, and pressure reversed the effect of this anesthetic on the lipid as seen by reversal of the changes in lineshape and the measured order parameter.

Similarly, high pressure ^{31}P-NMR was used in our laboratory (Peng and Jonas, 1992) to investigate the effects of pressure on the structure and dynamics of the phosphatidylcholine headgroup in multilamellar aqueous dispersions of

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DPPC with and without the positively charged form of TTC. The ^{31}P chemical shift anisotropies, $\Delta\sigma$, and the ^{31}P spin-lattice relaxation times, T_1, were measured as a function of pressure from 1 bar to 5 kbar at 50°C both for pure DPPC and DPPC/TTC bilayers. This pressure range permitted us to explore the headgroup behavior in Gel I ($P_{\beta'}$), Gel II ($L_{\beta'}$), Gel III, Gel X, and the interdigitated, Gel i, phases. For pure DPPC bilayers, pressure had an ordering effect on the phospholipid headgroup within the same phase and induced an interdigitated G_i gel phase that was formed between the Gel I ($P_{\beta'}$) and Gel II ($L_{\beta'}$) phases. The ^{31}P spin-lattice relaxation time measurements showed that the main phase transition (LC to Gel I), was accompanied by a transition between the fast and slow correlation time regimes. Axially symmetric ^{31}P-NMR lineshapes were observed at pressures up to 3 kbar but changed to characteristic axially asymmetric rigid lattice lineshapes at higher pressures (3.1–5.1 kbar).

In the present study, the ^{2}H-NMR lineshapes and relaxation times T_1 and T_2, of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine selectively deuterated at (2′,2′-^{2}H), (9′,9′-^{2}H), and (13′,13′-^{2}H) on both chains were measured as a function of pressure from 1 bar to 5 kbar at 50°C. As shown in our previous study (Peng and Jonas, 1992), this pressure range permits us to explore the phase behavior of DPPC from the liquid-crystalline (LC) phase through various gel phases such as the Gel I ($P_{\beta'}$), Gel II ($L_{\beta'}$), Gel III, Gel X, and the interdigitated gel phase, Gel i. The main goals of this study were as follows: (1) to determine the effects of pressure on deuterium lineshapes and relaxation times T_1 and T_2 of the different segments of DPPC; (2) to obtain order parameters as a function of pressure in the LC phase; (3) to determine pressure effects on the first moment $M1$, for the various high pressure gel phases; (4) to compare the motional behavior of the different chain segments and their response to pressure in the different phases; (5) to determine whether changing S_{CD} by increasing pressure in the LC phase yields linear T_1^{-1} vs. S_{CD}^2 and T_2^{-1} vs. S_{CD}^2 relationships for the different deuterated segments studied in multilamellar DPPC dispersions.

MATERIALS AND METHODS

Materials

The three different deuterated forms of DPPC: 1,2-dipalmitoyl(2′,2′-d4)--*sn*-glycero-3-phosphatidylcholine, 1,2-dipalmitoyl(9′,9′-d4)-*sn*-glycero-3-phosphatidylcholine, and 1,2-dipalmitoyl(13′,13′-d4)-*sn*-glycero-3-phosphatidylcholine were synthesized by Avanti Polar Lipids (Birmingham, AL). The sample purity was checked by thin layer chromatography using chloroform-methanol-water (65:25:4), v/v) as the solvent system before and after the experiments, and a single spot was found. The deuterium-depleted water used in the experiments was purchased from Aldrich Chemical Co. (Milwaukee, MI).

Sample preparation

The selectively deuterated DPPC samples were dispersed in deuterium-depleted water above the main phase transition temperature (>41°C). The hydrated samples were subject to extensive vortexing until homogeneous, milky white dispersions were formed. The resulting samples were transferred into NMR sample cells. The final concentrations of DPPC in the dispersions were approximately 35% by weight. The samples were not deoxygenated because of the short relaxation times of the samples. In addition, no differences in relaxation times were found between the deoxygenated and nondeoxygenated samples as reported by others (Brown et al., 1979; Mantsch et al., 1977).

NMR spectroscopy

^{2}H-NMR experiments were carried out on a home-built NMR spectrometer with a wide-bore (130 mm) Oxford 4.2 T superconducting magnet. The spectrometer is interfaced to a GE 293D pulse programmer and a GE/Nicolet 1280 computer system. The ^{2}H-NMR spectra were recorded at a 27.6-MHz spectrometer frequency. The pressure-transmitting fluid, CS_2, was used inside the titanium high pressure vessel, where the sample was isolated from CS_2 by a movable Teflon piston. An ethylene glycol and water mixture was circulated around the high pressure vessel to control the temperature. The sample temperature was measured through a copper-constantan thermocouple inside the vessel. Temperature and pressure were stable to within ±0.2°C and ±20 bar, respectively. The pressure range was from 1 bar up to 5 kbar, covering the liquid-crystalline range and various high pressure gel phases. The samples were allowed to equilibrate after each pressure change for 1 h within the same phase and at least 2 h between different phases. The NMR probe was retuned after each pressure measurement.

^{2}H-NMR spectra were obtained using various multipulse sequences. The typical pulse sequences are listed below:
The quadrupole echo sequence:

$$(\pi/2)_x - t_1 - (\pi/2)_y - t_2 - \text{echo};$$

The inversion recovery sequence:

$$(\pi)_{\text{comp}} - \tau - (\pi/2)_x - t_1 - (\pi/2)_y - t_2 - \text{echo}$$

In these sequences, the delay times t_1 and t_2 were chosen according to $t_d < t_1(t_2) \ll T_2$ (t_d is the dead time of the receiver, and T_2 is the transverse relaxation time), the subscripts x and y refer to the phases of various pulses differing by $\pi/2$ from each other. The typical values for t_1 and t_2 were in the range of 30–50 μs. The π and $\pi/2$ pulses in the above sequences were replaced by composite pulses to compensate for B_1 field inhomogeneities and pulse length inaccuracy. The composite pulses (Levitt et al., 1984) were $(3\pi/4)_x(\pi/2)_{-x}(\pi/4)_x$ or $(\pi/2)_{-x}(\pi)_x(\pi/2)_{-x}(3\pi/4)_x(\pi/4)_{-x}$ for the composite $\pi/2$ pulse and $(\pi/2)_x(\pi)_y(\pi/2)_x$ for the composite π pulse. An appropriate phase cycling scheme was used for the above pulse sequences, and quadrature phase detection was used for all experiments. Because of the finite pulse length, the spectra in the gel phases are very broad; therefore, we were unable to satisfy the condition $\omega_Q t_p \geq 1$ for a $\pi/2$ pulse, where ω_Q is the quadrupole splitting and t_p is the pulse length. To prevent spectral distortion, two $\pi/2$ pulses in the quadrupole echo sequence were replaced by two $\pi/4$ pulses in the various gel phases (Davis, 1979).

All spectra were recorded with a spectral width of 150 kHz and data size of 2K points. The $\pi/2$ pulse length was 6 μs, and line broadening was 50 Hz for the LC phase and 300 Hz for the gel phases. A total of 8,000–40,000 scans were accumulated depending on the sensitivity of the samples; the recycle delay was chosen to be greater than $5T_1$ for a full recovery of the magnetization. All FIDs were left-shifted to the echo maximum, and the data were transformed starting right on the top of the echo.

The quadrupole splitting values ($\Delta\nu_Q$) were obtained using the quadrupole echo sequence. The spin-lattice relaxation times (T_1) were measured from the inversion recovery sequence; the delay time τ was varied depending on the T_1 values of the samples. The spin-spin relaxation times (T_2) were determined using the quadrupole echo sequence with varying delay times t_1 and t_2 depending on the T_2 values of different samples.

RESULTS

Lineshape studies

^{2}H-NMR experiments have been carried out at various pressures at 50°C because at this temperature one can access various gel phases by increasing pressure (Driscoll et al., 1991; Peng and Jonas, 1992).

Figs. 1–3 show ^{2}H-NMR spectra of 1,2(2′,2′-^{2}H)DPPC, 1,2(9′,9′-^{2}H)DPPC, and 1,2(13′,13′-^{2}H)DPPC, respectively, in various phases. The lineshapes in the liquid-crystalline phase at 1–500 bar exhibit the characteristic axially symmetric powder pattern. These spectra have well defined powder patterns with sharp edged peaks and shoulders at about twice the frequency difference between the edge and the center of the spectrum. Fig. 1 shows that there are three quadrupole splittings for 1,2(2′,2′-^{2}H)DPPC in the LC phase. The largest quadrupole splitting is observed for the *sn*-1-chain deuterons, and the smaller two belong to the two nonequivalent deuterons of the *sn*-2 chain as described by Seelig and Seelig (1974). For 1,2(9′,9′-^{2}H)DPPC, one finds only one residual quadrupole splitting at 1–250 bar but two residual splittings from 375 to 500 bar, whereas for 1,2-(13′,13′-^{2}H)DPPC there exist two residual quadrupole splittings throughout the LC phase. As pressure is increased to 750 bar where the system enters into the Gel I phase, the sharp edges for all three samples disappear. As pressure is further increased up to the interdigitated Gel i gel phase, the intensities of the parallel edges of the spectra increase and then decrease again in the chain-tilted Gel II phase. The intensities of the parallel edges of the spectra increase progressively with increasing pressure. In the closely packed Gel X phase, prominent intensities at the outer edges are observed.

Because the residual quadrupole splittings can be determined unambiguously in the LC phase, the C-D bond order parameters S_{CD} can be calculated according to (Seelig, 1977)

$$\Delta\nu_{\mathrm{Q}} = \frac{3}{4}\left(\frac{e^2qQ}{h}\right)S_{\mathrm{CD}} \tag{1}$$

$$S_{\mathrm{CD}} = \tfrac{1}{2}\langle 3\cos^2\theta - 1\rangle \tag{2}$$

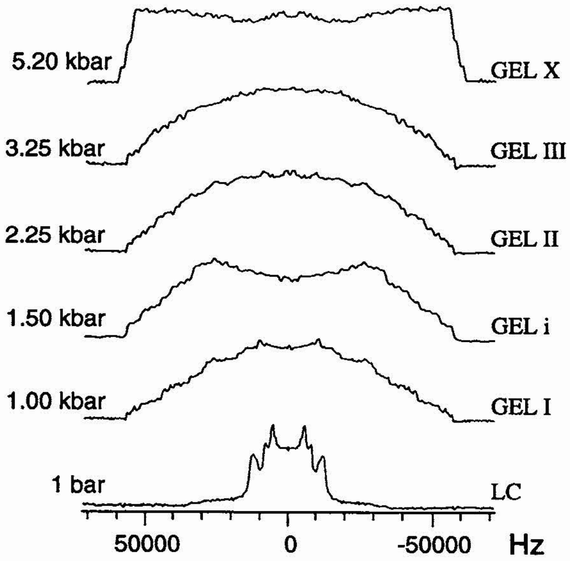

FIGURE 1 ^{2}H-NMR spectra of 1,2(2′,2′-^{2}H)DPPC mutilamellar dispersions at 50°C and different pressures.

where, (e^2qQ/h) is the static quadrupole splitting constant that has been determined to be 168 kHz for paraffinic CD bonds (Burnett and Muller, 1971; Davis and Jeffrey, 1977), and θ is the angle between the CD bond vector and the bilayer normal. However, in the gel phase the sharp powder pattern disappears; therefore, to estimate the ordering effect of pressure on the various chain segments, we measured the first moment ($M1$) of the spectra. Fig. 4 shows the measured $M1$ values as a function of pressure for the three samples. Inspection of the figure indicates that the values of $M1$ change markedly at the different phase transitions.

Relaxation studies

To understand better the dynamic properties of the DPPC bilayers, we measured the spin-lattice relaxation times (T_1) and spin-spin relaxation times (T_2). These relaxation times are listed in Tables 1–3 and are also shown in Figs. 5 and 6 as a function of pressure.

The T_1 values in the LC phase were measured from the decay of the peak intensity of the spectrum at the maximum echo. No distinct orientation dependence of T_1 values across the echo spectra was observed, in agreement with results reported by other authors (Brown and Davis, 1981; Perly et al., 1985). In the LC phase, two T_1 values were obtained for the 1,2-(2′,2′-^{2}H)DPPC samples at each pressure. A larger value was found for the *sn*-1-chain deuterons, whereas a smaller value was observed for the two magnetically nonequivalent deuterons of the *sn*-2 chain (Seelig and Seelig, 1974). For the other two samples, only average T_1 values were determined because of limited resolution. As indicated in Fig. 5, for each sample, there are three discontinuities

TABLE 1 Experimental first moments, spin-lattice relaxation times, and spin-spin relaxation times for 1,2-(2′,2′-^{2}H)DPPC

Phase	P (kbar)	$M1$ (10^5 Hz)	T_1 (ms)*	T_2 (μs)*
LC	0.001	0.691	13.8,‡ 17.1§	186
	0.125	0.743	12.1,‡ 15.6§	164
	0.250	0.802	10.5,‡ 14.0§	153
	0.375	0.833	8.85,‡ 12.9§	131
	0.500	0.871	7.43,‡ 11.5§	126
Gel I	0.750	1.05	14.8	23.1
	1.000	1.13	11.0	42.7
	1.300	1.23	8.25	115
Gel i	1.500	1.35	12.1	78.1
	1.750	1.39	10.3	125
	2.000	1.41	8.12	148
Gel II	2.250	1.31	4.80	82.1
	2.500	1.32	7.54	118
	2.750	1.34	19.1	155
Gel III	3.250	1.37	17.6	190
	3.700	1.41	7.83	228
	4.200	1.45	8.51	245
Gel X	4.500	1.56	9.02	187
	4.800	1.65	10.1	165
	5.200	1.72	10.8	154

*In the LC phase, different T_1 values are determined for different chains. However, the same T_2 values are obtained for two chains.

‡SN-2 chain.

§SN-1 chain.

TABLE 2 Experimental ^{2}H-NMR first moments, spin-lattice relaxation times, and spin-spin relaxation times for 1,2-(9′,9′-^{2}H)DPPC

Phase	P (kbar)	$M1$ (10^5 Hz)	T_1 (ms)*	T_2 (μs)*
LC	0.001	0.582	33.2	287
	0.125	0.650	32.5	266
	0.250	0.683	30.1	260
	0.375	0.731	27.2	254
	0.500	0.780	25.8	245
Gel I	0.750	1.25	33.1	78.5
	1.000	1.29	25.5	138
	1.300	1.37	20.2	185
Gel i	1.500	1.51	28.9	69.5
	1.750	1.54	24.8	103
	2.000	1.56	18.9	135
Gel II	2.250	1.42	14.5	58.2
	2.500	1.46	34.7	93.7
	2.750	1.48	47.8	128
Gel III	3.250	1.56	42.1	145
	3.700	1.60	16.8	192
	4.200	1.69	11.7	214
Gel X	4.500	1.81	11.0	175
	4.800	1.86	11.5	161
	5.200	1.89	12.7	150

*Only single T_1 and T_2 values are determined for different chains because of limited resolution.

TABLE 3 Experimental ^{2}H-NMR first moments, spin-lattice relaxation times, and spin-spin relaxation times for 1,2-(13′,13′-^{2}H)DPPC

Phase	P (kbar)	$M1$ (10^5 Hz)	T_1 (ms)*	T_2 (μs)*
LC	0.001	0.420	65.9	414
	0.125	0.451	62.4	396
	0.250	0.505	60.3	392
	0.375	0.523	56.0	377
	0.500	0.557	54.1	363
Gel I	0.750	1.10	59.5	123
	1.000	1.21	55.2	185
	1.300	1.32	52.0	225
Gel i	1.500	1.44	59.2	55.3
	1.750	1.46	53.1	81.1
	2.000	1.49	45.6	115
Gel II	2.250	1.35	41.0	43.1
	2.500	1.41	57.5	70.3
	2.750	1.42	64.5	95.2
Gel III	3.250	1.49	61.7	113
	3.700	1.54	30.1	145
	4.200	1.59	15.8	187
Gel X	4.500	1.74	14.5	160
	4.800	1.79	15.0	152
	5.200	1.83	14.1	141

*Only single T_1 and T_2 values are determined for different chains because of limited resolution.

or break points in T_1 values that occur at phase transitions LC/Gel I, Gel I/Gel i, and Gel i/Gel II, respectively. In addition, one local maximum occurs at phase transition Gel II/Gel III and another local minimum occurs near phase transition Gel III/Gel X. As a function of pressure, all three samples have almost the same pressure dependence, with the order of magnitude of T_1 values being C-13 > C-9 > C-2 at each pressure in each phase. The T_1 values decrease with increasing pressure in each phase except in the Gel II phase, where the opposite dependence is observed.

As shown in Fig. 6, the T_2 values for the three samples in the LC and Gel I phases vary in magnitude from C-13 > C-9 > C-2 at each pressure. However, in the other high pressure gel phases, from Gel i to Gel X phase, the order of T_2 values is inverted with C-2 > C-9 > C-13. From Fig. 6 one can see that the T_2 values decrease as pressure is increased in the LC and Gel X phases, whereas they increase with increasing pressure in the other gel phases. In addition, a local maximum was found near the phase transition between Gel III and Gel X phases.

DISCUSSION

Lineshape studies

As we discussed in our earlier studies (Driscoll et al., 1991; Peng and Jonas, 1992) the use of pressure as an experimental variable allows us to explore the complex barotropic phase behavior of DPPC. The deuterium lineshapes at different pressures at 50°C (Figs. 1–3) reflect the phase transitions and the changes in the order and mobility of DPPC molecules as pressure increases.

In the LC phase, three different quadrupole splittings, with the largest one from the *sn*-1 chain and smaller two from the *sn*-2 chain, indicate differences in mobility for both chains at the C-2 position (Seelig and Seelig, 1974). One quadrupole splitting observed for C-9 position at 1–250 bar and two quadrupole splittings at 370 and 500 bar suggest a similar order and mobility for the *sn*-1 and the *sn*-2 chains at the C-9 position at low pressures but different behavior at higher pressure as the chains pack closer together. Two quadrupole

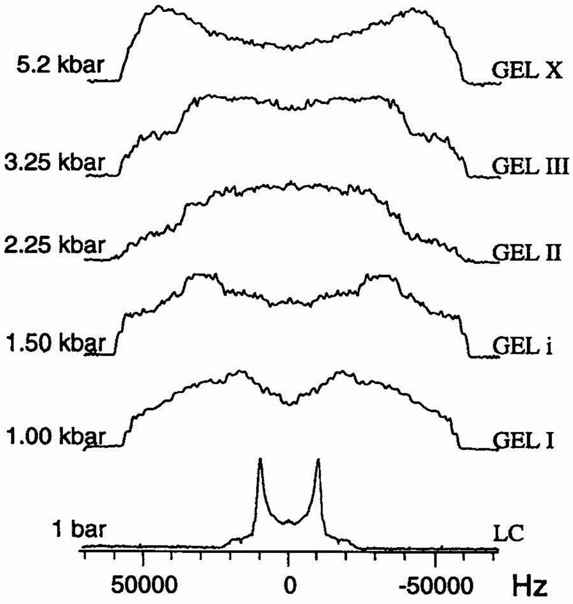

FIGURE 2 ^{2}H-NMR spectra of 1,2(9′,9′-^{2}H)DPPC mutilamellar dispersions at 50°C and different pressures.

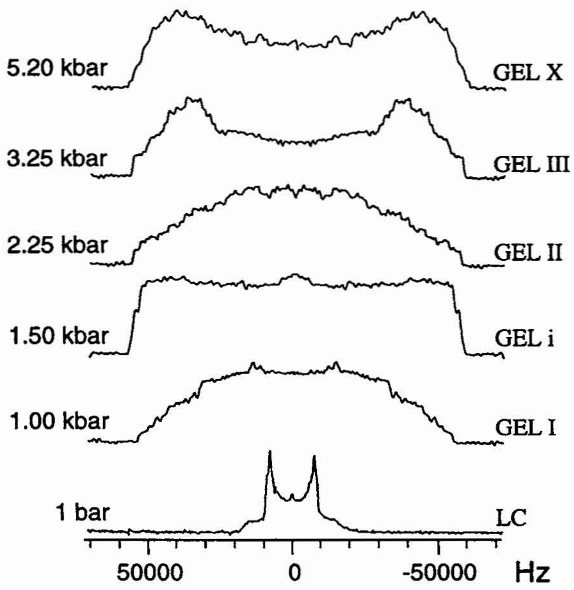

FIGURE 3 ^{2}H-NMR spectra of 1,2(13′,13′-^{2}H)DPPC mutilamellar dispersions at 50°C and different pressures.

splittings were observed for the C-13 sample, indicating differences in segmental mobility and order for the two chains throughout the LC phase. The sharp powder spectra in the LC phase for all three samples indicate rapid inter- and intramolecular motions, including *trans-gauche* isomerizations, and motions around the main molecular axis.

Interpretation of the deuterium lineshapes in the various gel phases is only phenomenological because of the complexity of computer simulations of the spectra as described by Kothe and co-workers (Meier et al., 1986, 1988) for the case of 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine. Therefore, in the following discussion we only highlight the qualitative features of the deuterium lineshapes in the high pressure gel phases. Our results suggest significant axial motions in the interdigitated Gel i and Gel III phases for all three segments. No variations of lineshape with pulse spacing are observed in the Gel I and Gel II phases, in agreement with results on DPPC bilayers at 18 and 0°C by Ruocco et al. (1985) and Davis (1983).

In the Gel I phase, the abrupt onset of a very short T_2 and the broader splittings of the LC-like components indicate the presence of an exchange process due to the slow lateral diffusion between the lipid molecules in LC domains and the gel domains. It was found by Davis and Jeffrey (1977) that the quadrupole splitting for the all-*trans* conformation of ethylene in a hydrocarbon chain is 126 kHz (spectral peaks located at ±63 kHz). Therefore, the appearance of spectral intensity at the shoulders near ±60 kHz for all samples indicates significant increases in the population of *trans* conformers. In the Gel I phase, as well as in all the other gel phases, the C-9 segment has the largest $M1$ values, implying that the middle parts of the chains are most rigid. IR studies on chain-deuterated DPPC (Cameron et al., 1981) also indicated that the most rigid packing is in the central parts of the chains. However, Fig. 4 shows the unexpected result that $M1$ values of the C-2 segment are smaller than those of the C-9 and C-13 segments in the gel phases. A smaller $M1$ value of C-2 segment compared with other segments in the gel phase was also found in palmitate bilayers; to explain this observation, a conformational change in the C-2 segment was proposed (Davis and Jeffrey, 1977; Abdolall et al., 1977). Moreover, ^{13}C-NMR studies on DPPC and other phospholipids have indicated conformational changes at the carbonyl group of the *sn*-2 chain at the main thermotropic phase transition (Wittebort et al., 1981, 1982; Blum et al., 1982); and high pressure IR studies on DPPC bilayers demonstrated the existence of hydrogen bonding at the carbonyl group of the *sn*-2 chain (Wong and Mantsch, 1988). In view of these results, we propose that there may be a conformational change of the C-2 segment resulting in smaller $M1$ values.

The interdigitated Gel i gel phase is formed as pressure is increased above the Gel I phase. A significant increase in spectral intensity near ±60 kHz shows that most of the segments are in the *trans* conformation. In the Gel i phase, it is expected that conformational freedom as well as the inter- and intra-molecular motions of the acyl chains should be reduced because of crowding in the chain region. This is indeed reflected in the higher $M1$ values in this interdigitated phase compared with those in the Gel I and Gel II phases (Fig. 4), which indicates that the chain packing is more rigid in the Gel i phase than in the adjacent gel phases. Our results are in good agreement with previous reports on the Gel i phase by Braganza and Worcester (1986a, b).

On passing from the interdigitated Gel i phase to the Gel II phase, the shoulder intensities near ±60 kHz decline, but intensities in the central portion of the spectrum increase as shown in Figs. 1–3. Concurrently, the measured $M1$ values drop abruptly, indicating a significant increase in chain flexibility and increased probability of *gauche* conformations.

In both the Gel III and Gel X phases, the shoulder intensities increase continuously as pressure is increased. At the

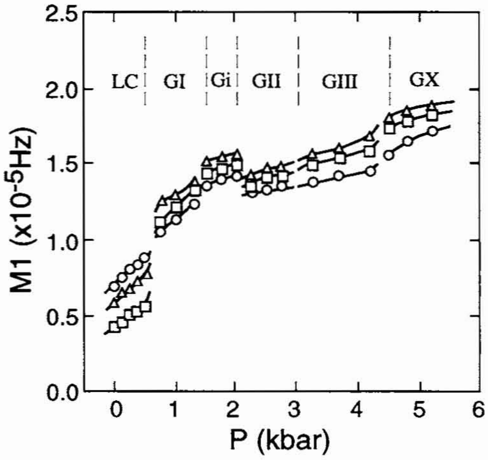

FIGURE 4 Pressure dependence of the first moment, $M1$, for 1,2(2′,2′-^{2}H)DPPC (○), 1,2(9′,9′-^{2}H)DPPC (△), and 1,2(13′,13′-^{2}H)DPPC (□) samples at 50°C.

same time, *M*1 values increase for all of the segments, suggesting a gradual increase in chain rigidity. The prominent intensities at the shoulders in the Gel X phase indicate that the chains are almost completely in the *trans* conformation. Also, the absence of perpendicular edges in the spectra indicates that the axial diffusion has stopped on the ^{2}H-NMR time scale in the Gel X phase.

Careful examination of all of the spectra in the various gel phases indicates that the shoulders occur at values smaller than ±60 kHz, which is the typical value of all of the *trans* conformations in the hydrocarbon chain. Therefore, there are still some *gauche* conformations in the various gel phases, including the Gel X phase.

Relaxation studies

In this study, T_1 and T_2 of relaxation times have been measured using multipulse sequences to detect motions with different frequencies. Because many types of motions are involved in this system, it is not surprising to find a complicated relaxation behavior as shown in Figs. 5 and 6. A quantitative evaluation of theses motional rates requires comprehensive computer simulation and appropriate molecular modeling (Meier et al., 1986), which are beyond the scope of this study. In the following paragraphs, we discuss the main features of the relaxation results in a qualitative way.

The spin-lattice relaxation time, T_1, is sensitive to motions with a correlation time τ_c near ω_0^{-1}; therefore, motions with correlation times in the range from 10^{-8}-10^{-11} s are accessible from spin-lattice relaxation measurements. In the LC phase, the different T_1 values observed for deuterons on the *sn*-1 and *sn*-2 chains (Table 1) indicate clearly that the motional states of the C-2 segment of the *sn*-1 chain are different from those of the *sn*-2 chain. The order of T_1 values in the LC phase is C-13 > C-9 > C-2, indicating a greater motional freedom toward the end of the acyl chain. The three discontinuities at phase transitions LC/Gel I, Gel I/Gel i, and Gel i/Gel II indicate abrupt changes in rates for different modes of motion among these phases. In the Gel I and Gel i phases, the largest T_1 for the C-13 segment shows that the C-13 segment is the most mobile in the fast correlation time region (10^{-8}-10^{-11} s). As illustrated in Fig. 5, the T_1 values increase with pressure in the Gel II phase, reach a maximum at the phase transition between Gel II/Gel III, and then decreases in the Gel III phase. A further increase in pressure results in a local minimum near the phase transition between Gel III/Gel X and a slight increase in the Gel X phase. All three segments approach a common T_1 value in the Gel X phase, suggesting a common motional mode in the Gel X phase that, most likely, is the *trans-gauche* isomerization. Inspection of Fig. 5 shows that the order of magnitude of T_1 is C-13 > C-9 > C-2 in all of the phases, maintaining the same order of motional rates for the three segments.

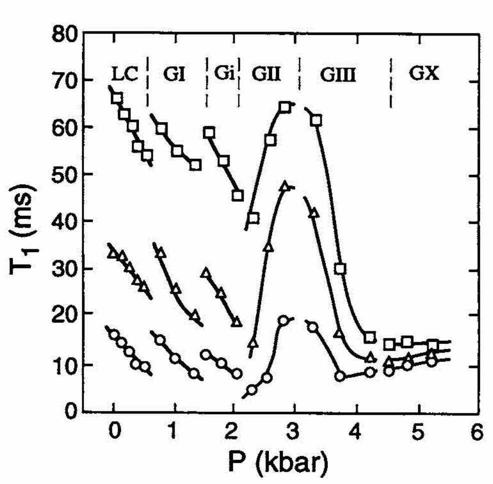

FIGURE 5 The spin-lattice relaxation time, T_1, as a function of pressure for 1,2(2′,2′-^{2}H)DPPC (○), 1,2(9′,9′-^{2}H)DPPC (△), and 1,2(13′,13′-^{2}H)-DPPC (□) samples at 50°C.

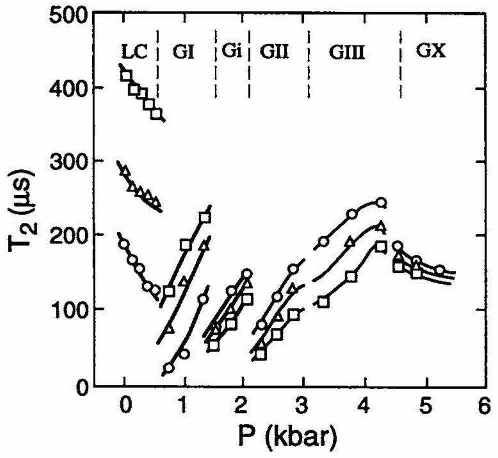

FIGURE 6 The spin-spin relaxation time, T_2, as a function of pressure for 1,2(2′,2′-^{2}H)DPPC (○), 1,2(9′,9′-^{2}H)DPPC (△), and 1,2(13′,13′-^{2}H)DPPC (□) samples at 50°C.

In comparison with T_1, the spin-spin relaxation time T_2 is more sensitive to motions with correlation times near $(e^2qQ/h)^{-1}$. Therefore, T_2 measurements provide information on motions with correlation times in the intermediate to slow range in (10^{-4}-10^{-8} s). In the LC phase, the same T_2 values are obtained for the C-2 segment of the two chains, indicating the same segmental motional rate in the intermediate correlation time region (See Tables 1–3). The variation of T_2 with chain position represents different motional rates for different segments in the LC phase. The order of magnitude of T_2 is C-13 > C-9 > C-2, indicating the greatest mobility for the C-13 segment, in agreement with the T_1 results. In addition, the T_2 values decrease with increasing pressure in the LC phase, indicating a motionally narrowed regime. Much reduced T_2 values are observed just below the main phase transition, resulting in a significant echo intensity loss of the spectra. In the Gel I phase, the T_2 values increase with increasing pressure, indicating a slow correlation time regime. The same trend was also observed in DMPC bilayers (Mayer et al., 1988) at the transition between the LC and the gel phases. Fig. 6 shows three discontinuities in T_2 values at the phase transitions for LC/Gel I, Gel I/Gel i, and Gel i/Gel II, indicating different dynamic properties for the system in the different gel phases. The T_2 values increase with pressure in the Gel I, Gel i, Gel II, and Gel III phases. In addition, there is a local maximum near the phase transition between Gel III and Gel X. A further increase in pressure causes a decrease in T_2 values in the Gel X phase where all segments approach the same T_2 value, indicating a common motional mode in the Gel X phase.

Fig. 6 also shows a variation of T_2 values with the chain position in all of the phases. Our results are different from those for DMPC bilayers obtained by Mayer et al. (1988), where the same T_2 value was observed in the gel phases for all chain positions except for the terminal methyl group. The differences reflect the different dynamic properties of these two phospholipids as well as differences in the dynamics of the thermotropic and barotropic gel phases. In contrast to the LC and Gel I phases, the order of T_2 values becomes C-2 > C-9 > C-13 for Gel i through Gel X phase, possibly reflecting slow axial motions and the greater range of motions of the C-2 segment as shown in Fig. 4.

The availability of experimental pressure dependencies for T_1, T_2, and S_{CD} as determined for the selectively deuterated DPPC molecules in the LC phase, allowed us to test whether linear T_1^{-1} vs. S_{CD}^2 and T_2^{-1} vs. S_{CD}^2 relationships (Brown, 1984; Watnick et al., 1990) also hold at changing pressures. One should point out that there is still a difference of opinion whether this linear relationship signifies collective motions for various chain segments (Brown, 1984; Rommel et al. 1988; Bloom et al. 1991; Watnick et al. 1990). In our experiments, a linear relationship between T_1^{-1} and S_{CD}^2 is indeed observed. We find in Fig. 7 that all data points fall on the same straight line for 1,2(9′,9′-^{2}H)DPPC and 1,2-(13′,13′-^{2}H)DPPC at all pressures in the LC phase at 50°C (T_1^{-1} = 738.8 s^{-1} S_{CD}^2 +1.59 s^{-1}). In contrast, the T_1^{-1} vs. S_{CD}^2 for C-2 *sn*-2 deuterons and *sn*-1 deuterons do not fall on the same straight line and have an intercept value that is nonphysical (<0). Similarly to Fig. 7, Fig. 8 shows a linear relationship between T_2^{-1} and S_{CD}^2. The data for C-9 and C-13 segments fall on the same straight line with the following equation: $T_2^{-1} = 5.329 \times 10^4\ \mathrm{s}^{-1}\ S_{CD}^2 + 1480\ \mathrm{s}^{-1}$. Once again, there are two lines with different slopes and intercepts for the C-2 segments of the two chains, suggesting that the motions for the C-2 segments are not correlated with the other two segments. Apparently, the cooperative chain motions do not extend to the C-2 segment in contrast to the DMPC bilayers (Brown, 1984). However, our results are in agreement with the conclusions of Brown (1984) and Watnick et al. (1990) that the cooperative motions extend from at least C-9 toward the end of the acyl chains. The chain carbons near the glycerol backbone may act as anchor groups in DPPC bilayers and do not obey the common linear relationship observed for the 9 and 13 positions.

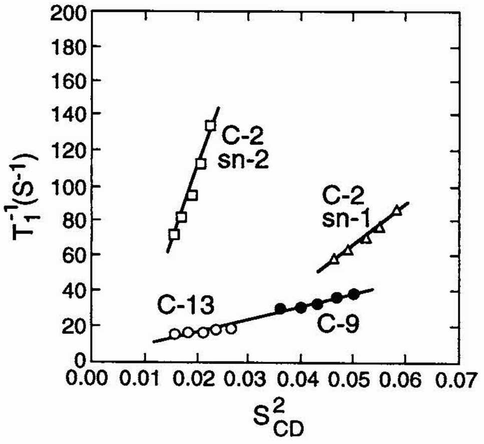

FIGURE 7 The spin-lattice relaxation time, T_1, as a function of the order parameter, S_{CD}, at 50°C: (○) 1,2(9′,9′-^{2}H)DPPC and (●) 1,2(13′,13′-^{2}H)-DPPC; (△) C-2 deuterons of the *sn*-1 chain in 1,2(2′,2′-^{2}H)DPPC; (□) C-2 deuterons of the *sn*-2 chain in 1,2(2′,2′-^{2}H)DPPC.

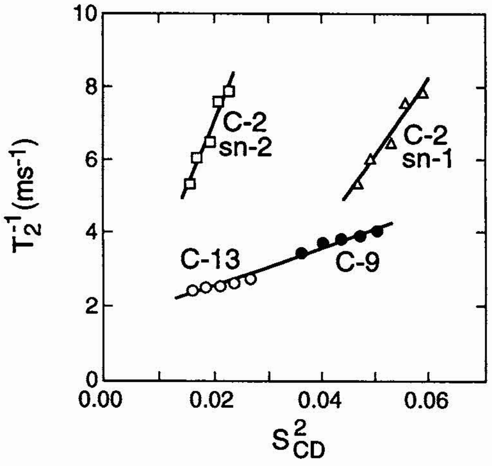

FIGURE 8 The spin-spin relaxation time, T_2, as a function of the order parameter, S_{CD}, at 50°C: (○) 1,2(9′,9′-^{2}H)DPPC and (●) 1,2(13′,13′-^{2}H)-DPPC; (△) C-2 deuterons of the *sn*-1 chain in 1,2(2′,2′-^{2}H) DPPC; (□) C-2 deuterons of the *sn*-2 chain in 1,2(2′,2′-^{2}H)DPPC.

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