Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926090

Deuteron N.M.R. relaxation studies of phospholipid membranes

C. Mayer[a]; K. Müller[a]; K. Weisz[a]; G. Kothe[a]
[a] Institut für Physikalische Chemie, Universität Stuttgart, Stuttgart 80, F.R. Germany

To cite this Article Mayer, C. , Müller, K. , Weisz, K. and Kothe, G.(1988) 'Deuteron N.M.R. relaxation studies of phospholipid membranes', Liquid Crystals, 3: 6, 797 — 806
To link to this Article: DOI: 10.1080/02678298808086536
URL: http://dx.doi.org/10.1080/02678298808086536

LIQUID CRYSTALS, 1988, VOL. 3, NOS. 6 AND 7, 797–806

Deuteron N.M.R. relaxation studies of phospholipid membranes

by C. MAYER, K. MÜLLER, K. WEISZ and G. KOTHE
Institut für Physikalische Chemie, Universität Stuttgart, Pfaffenwaldring 55, D-7000 Stuttgart 80, F.R. Germany

Bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), specifically deuteriated at various positions of the *sn*-2-chain, have been studied by N.M.R. relaxation methods. Analysis of the experiments, employing a density matrix treatment based on the stochastic Liouville equation, provides new information about the dynamic organization of the different membrane phases (liquid-crystalline, intermediate and gel phases). The complex molecular dynamics are characterized by a super-position of inter- and intramolecular motions, comprising overall reorientation of phospholipid molecules and trans-gauche isomerization of individual chain segments. In addition, there is evidence for two-site rotational jumps of the *sn*-2-chains in the plane of the membrane. The results clearly demonstrate the particular advantage of N.M.R. relaxation studies in characterizing complex chemical and biological systems.

1. Introduction

Phospholipid membranes have been studied by a variety of spectroscopic techniques. Among these, pulsed deuteron (^{2}H) N.M.R. has played an important role in the elucidation of the dynamic organization of these systems [1–9]. By using different pulse sequences, the various molecular motions can be differentiated over a wide dynamic range not accessible by any other spectroscopic technique [4, 8]. In addition, various types of molecular order, modulated by the different motions, are similarly discriminated [4, 8].

Despite the fact that ^{2}H relaxation experiments are now readily performed, detailed quantitative analysis has been rare. This is mainly due to the fact that the phospholipid phases cover a broad dynamic range and therefore require a comprehensive N.M.R. model considering both restricted solid state and continuous liquid state motions. In this paper we present ^{2}H relaxation studies of macroscopically unoriented bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), specifically deuteriated at the 2-, 6-, 13- and 14-positions of the *sn*-2-chain. Analysis of the various experiments is achieved by employing a relaxation model based on the stochastic Liouville equation. Detailed information about molecular order and dynamics is obtained. The results, referring to three different membrane phases, are discussed in relation to other studies of the molecular properties of membranes.

2. Experiments and methods

2.1. *Syntheses and preparations*

The syntheses of DMPC, specifically deuteriated at the 2-, 6-, 13- and 14-position of the *sn*-2-chain, are basically described elsewhere [10]. Samples were prepared from 50 wt % deuteriated phospholipid in deuterium depleted water. Calorimetric measurements (Perkin Elmer DSC 7) showed two phase transitions for the bilayer

samples: (i) a main transition at 297 K from the liquid-crystalline (L_α) to the intermediate ($P_{\beta'}$) phases; and (ii) a pretransition at 287 K from the intermediate to the gel phases ($L_{\beta'}$). Recently, a further transition to a subgel phase has been observed after incubation at 273 K for several days [11]. In order to prevent formation of this subgel phase, the samples were first heated to the L_α phase and then studied at descending temperatures.

2.2. *N.M.R. experiments*

^{2}H N.M.R. experiments were performed on Bruker CXP 300 and MSL 300 spectrometers at $\nu = 46{\cdot}1$ MHz ($B_0 = 7$ T), using quadrature detection and appropriate phase cycling schemes. Relaxation measurements were carried out applying quadrupole echo ($(\pi/2)_x - \tau_1 - (\pi/2)_y$), inversion recovery ($\pi - \tau_1 - (\pi/2)_x - \tau_2 - (\pi/2)_y$) and saturation recovery ($(\pi/2) - \tau_1 - (\pi/2)_x - \tau_2 - (\pi/2)_y$) sequences. The number of scans varied between 400 and 4000 depending on the particular experiment.

2.3. *Data analysis*

Analysis of the ^{2}H N.M.R. relaxation experiments was achieved using a dynamic N.M.R. model based on the stochastic Liouville equation [4, 8, 12]. Various adjustable parameters, characterizing molecular order and dynamics, enter the problem. In the specific case of lipid bilayers, both inter- and intramolecular motions are considered. The intermolecular motion is the motion of the lipid molecules as a whole. It is assumed that they undergo continuous, anisotropic diffusion within an ordering potential. The intramolecular motion consists of jumps between discrete conformations. The corresponding correlation times $\tau_{R\parallel}$, and $\tau_{R\perp}$ and τ_J specify the time scale of these motions.

Similarly, molecular order is described by contributions at different molecular levels. They account for orientational order of the lipid molecules with respect to a preferential local axis (director axis) and conformational order of the chain segments. The relevant quantities are denoted as orientational (S_{ZZ}) and segmental order ($S_{Z'Z'}$) parameters, respectively [8].

A Fortran program package was employed to analyse the ^{2}H N.M.R. experiments. Numerical solution of the stochastic Liouville equation was achieved using the Lanczos algorithm [13]. For large matrix dimensions ($N \geqq 1000$) the Lanczos algorithm was found to yield reliable numerical results with considerable reduction in computing time and computer storage requirements. Within the Redfield limit [14], analytical solutions [15, 16] were employed.

The analysis of relaxation experiments in terms of several simultaneously occurring motions requires a special strategy. Generally, each experiment defines a specific dynamic window in which molecular motions can be studied [8, 12]. For instance, the spin lattice relaxation times T_{1Z} (from inversion and saturation recovery sequences) are particularly sensitive to motions with correlation times $\tau_R \approx \omega_0^{-1}$. Thus, by employing a high magnetic field ($B_0 = 7$ T) fast molecular dynamics in the range 10^{-11} s to 10^{-7} s can be studied. In contrast, measurement of the spin–spin relaxation time T_{2E} (from quadrupole echo sequences) permits the study of much slower molecular motions. Since T_{2E} is most sensitive to motions with correlation times $\tau_R \approx (e^2qQ/\hbar)^{-1}$, quadrupole echo sequences offer a means to study molecular dynamics in the range $10^{-8}\,\text{s} < \tau_R < 10^{-4}\,\text{s}$.

Generally, the absolute value of the relaxation time at the minimum of the relaxation curve is highly indicative of the type of motion [12, 15]. Thus, by proper analysis, one motion can be extracted which dominates a particular relaxation experiment. Having extracted the dominant motion in one such region, its temperature dependence is extrapolated into other regions, where the situation is not so straightforward. The reliability of this procedure has, of course, to be checked by comparing the simulated relaxation curves with the corresponding experimental ones. The analysis is completed if all relaxation experiments can be simulated using the same set of parameters.

3. Results

Macroscopically unoriented bilayers of DMPC, specifically deuteriated at C-2, C-6, C-13 and C-14 of the *sn*-2-chain, were studied over a wide temperature range employing dynamic ^{2}H N.M.R. techniques. In figure 1 the spin–spin relaxation times T_{2E} of the DMPC membranes are plotted as a function of $1/T$. Generally, T_{2E} is defined as the time it takes for the quadrupole echo amplitude to decay to $1/e$ of its original value. This definition includes non-exponential decay, as observed for the powder samples, particularly at lower temperatures. Interestingly, the T_{2E} curves for the various ^{2}H labels are quite similar. In the L_α phase all T_{2E} values decrease with decreasing temperature. At the main transition T_{2E} drops abruptly by one order of magnitude. In the $P_{\beta'}$ phase T_{2E} first increases, reaching a local maximum just below the pretransition, then decreases and finally passes through a minimum at about 263 K. In the $P_{\beta'}$ and $L_{\beta'}$ phases the T_{2E} values for DMPC-2-d_2, DMPC-6-d_2 and

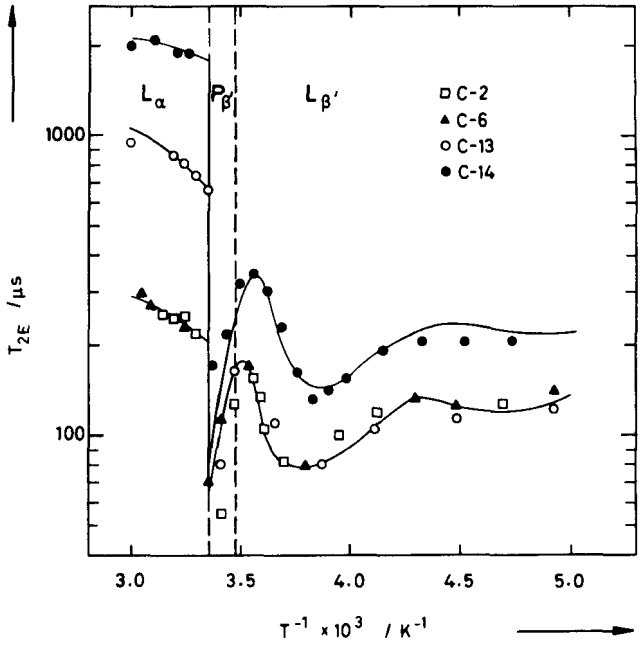

Figure 1. Temperature dependence of the ^{2}H spin–spin relaxation times, T_{2E}, of DMPC membranes, deuteriated at different positions of the *sn*-2-chain: (□) C-2, (▲) C-6, (○) C-13, (●) C-14. The solid lines represent best fit simulations using the parameters given in figures 3 and 4.

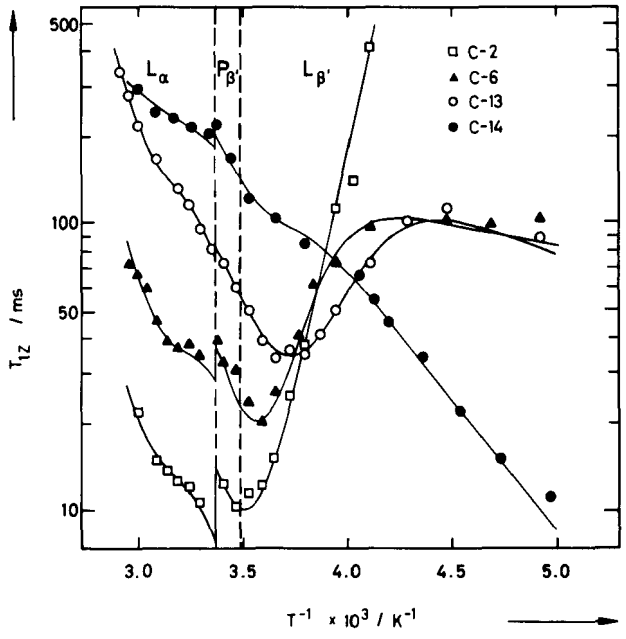

Figure 2. Temperature dependence of the ^{2}H spin lattice relaxation times T_{1Z} of DMPC membranes, deuteriated at different positions of the *sn*-2-chain: (□) C-2, (▲) C-6, (○) C-13, (●) C-14. The solid lines represent best fit simulations using the parameters given in figures 3 and 4.

DMPC-13-d_2 agree within experimental error, whereas higher values are observed for DMPC-14-d_3. This finding can be rationalized by fast methyl group rotation, which averages the quadrupole coupling constant to 1/3 of its original value [4, 8]. The similarity of the T_{2E} relaxation curves implies a common motional mode, which can be assigned to reorientation of lipid molecules as a whole ($\tau_{R\parallel}$, $\tau_{R\perp}$). The assignment is confirmed by lineshape simulations of partially relaxed quadrupole echo spectra [4, 8].

The spin lattice relaxation times T_{1Z}, shown in figure 2, were obtained from inversion recovery and saturation recovery sequences. They refer to DMPC-2-d_2, DMPC-6-d_2, DMPC-13-d_2 and DMPC-14-d_3 and extend over a wide temperature range. As before, these relaxation times are defined as the time it takes for the echo amplitude to decay to $1/e$ of its original value. We see that the temperature dependences for $T > 267$ K are quite similar. In the L_α phase all T_{1Z} curves decrease with decreasing temperature, exhibiting characteristic inflexions at approximately 323 K. At the main transition a small but significant increase of T_{1Z} is observed. On further cooling, all T_{1Z} values, except those of DMPC-14-d_3, pass through a common minimum at about 273 K. The further course depends on the labelled segment. The T_{1Z} values for DMPC-6-d_2 and DMPC-13-d_2 level off to a common plateau, while those of DMPC-2-d_2 continuously increase upon cooling. In contrast, T_{1Z} of DMPC-14-d_3 decreases, approaching a low temperature minimum at about 150 K (not shown) caused by methyl group rotation [8]. The most interesting result is the common T_{1Z} minimum at approximately 273 K. The measured T_{1Z} values at the minimum are not compatible with overall rotation or fluctuations of lipid molecules. Rather, they indicate two-site rotational jumps of the *sn*-2-chain in the plane of the membrane,

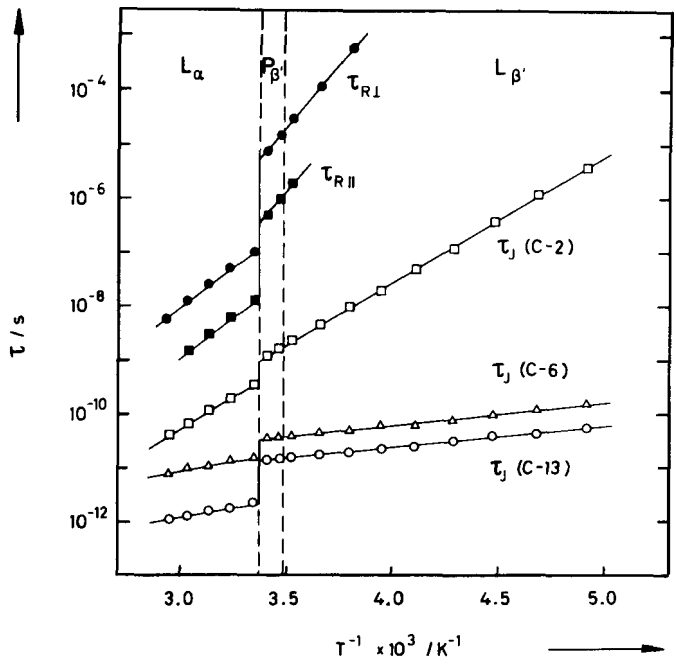

Figure 3. Arrhenius plot of various correlation times, characterizing the inter- and intramolecular motions of DMPC membranes: (■) overall rotation $\tau_{R\parallel}$, (●) overall fluctuation $\tau_{R\perp}$, trans-gauche isomerization τ_J at (□) C-2, (△) C-6 and (○) C-13 segments.

resulting from gauche–gauche isomerization at the C-2 segment [17]. Apparently, this motion affects all segments equally [8].

The observed relaxation curves were simulated, employing the N.M.R. model outlined previously. An iterative fit of several pulse dependent experiments for any given temperature provided reliable values for the simulation parameters, i.e. the rotational correlation times and the order parameters. The solid lines in figures 1 and 2 represent best fit simulations. They agree favourably with their experimental counterparts. Note that relaxation curves of four different label positions are reproduced by our model with the same set of simulation parameters. In the following we describe the results in more detail, treating the various simulation parameters separately.

In figure 3 the correlation times for various motions of DMPC membranes are plotted as a function of $1/T$. They refer to overall reorientation of lipid molecules ($\tau_{R\parallel}$, $\tau_{R\perp}$, full symbols) and trans–gauche isomerization of individual chain segments (τ_J, open symbols). The dashed lines indicate different phase transitions. We see that the Arrhenius plots are linear within a particular phase, showing discontinuities at the phase transitions. From the slopes of the straight lines, motional activation energies have been determined. The values of $60\,\text{kJ/mol} < E_{R\parallel}, E_{R\perp} < 90\,\text{kJ/mol}$ reflect the intermolecular character of these motions, representing reorientations of the lipid molecules as a whole. As expected, the activation energies for trans–gauche isomerization at C-6 and C-13 ($8\,\text{kJ/mol} < E_J < 15\,\text{kJ/mol}$) are substantially smaller. A value of $E_J = 45\,\text{kJ/mol}$, determined for C-2, corresponds to the particular isomerization process at this segment [17].

In the L_α phase the correlation times for overall reorientation range from 10^{-9}s to 10^{-7}s, exhibiting a constant anisotropy ratio of $\tau_{R\perp}/\tau_{R\parallel} = 10$. At the main

transition both motions slow down abruptly by nearly two orders of magnitude. In the $P_{\beta'}$ phase the situation is more complicated, as two motional components are now observed. One component shows similar intermolecular dynamics as observed in the L_α phase (overall rotations and fluctuations). For the second component only slow fluctuations with correlation times of $\tau_{R\perp} > 10^{-6}$ s can be detected. The fraction of the motional restricted component strongly increases with decreasing temperature. Thus, about 10 K below the pretransition, only the immobile component is left.

The open symbols in figure 3 indicate correlation times for intramolecular motions. They refer to trans–gauche isomerization at the C-2, C-6 and C-13 segments, respectively. We see that these motions are considerably faster, exhibiting correlation times of 10^{-12} s $< \tau_J < 5 \times 10^{-10}$ s in the L_α phase. Consequently, their effect on T_{1Z} is rather small. Note the pronounced mobility gradient along the hydrocarbon chains. Apparently, the rate of trans–gauche isomerization increases considerably (two orders of magnitude) from the C-2 to the C-13 segment. At the main transition there is a significant slowing down of the intramolecular motions. However, no indication of microscopic heterogeneity with respect to trans–gauche isomerization can be detected in the $P_{\beta'}$ phase.

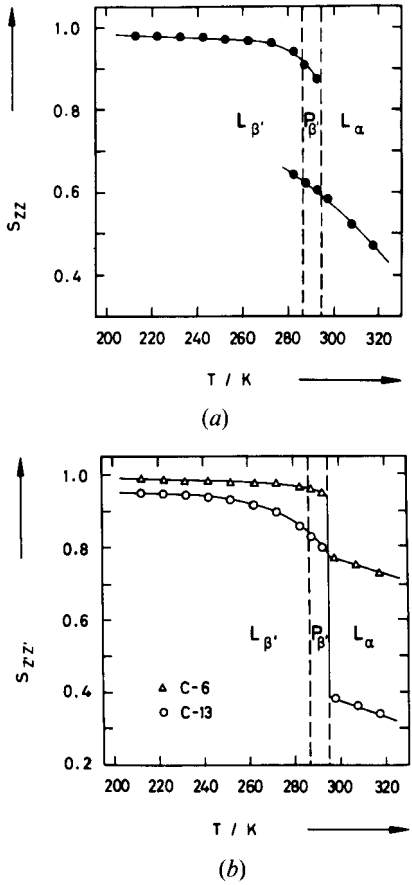

Figure 4. Temperature dependence of order parameters, characterizing the various phases of DMPC membranes. (*a*) Orientational order parameter S_{ZZ} of the lipid molecules; (*b*) segmental order parameter $S_{Z'Z'}$ at (△) C-6 and (○) C-13 positions.

The molecular order of phospholipid membranes comprises the orientational order of the lipid molecules and the conformational order of the various segments. In figure 4 the corresponding order parameters of DMPC bilayers are plotted as a function of temperature. In the L_α phase orientational order increases continuously with decreasing temperature, varying between $0{\cdot}4 < S_{ZZ} < 0{\cdot}6$ (see figure 4(*a*)). Below the main transition the situation is more complicated as two different components are observed in the quadrupole echo spectra. Appropriate simulation is only possible with two different order parameters, S_{ZZ} [4, 8]. The fraction of the higher ordered component increases significantly with decreasing temperature. As a result, below 277 K only the high order component is left.

Conformational order at the various chain segments is conveniently described in terms of segmental order parameters, $S_{Z'Z'}$ [8]. Within the limits of a completely disordered segment, all four tetrahedron sites are equally populated, resulting in an order parameter of $S_{Z'Z'} = 0$. At the other extreme, a fully extended chain is fixed to its all-trans conformation and the order parameter $S_{Z'Z'}$ becomes equal to one. In figure 4 (*b*) these segmental order parameters are plotted as a function of temperature. They refer to the C-6 and C-13 segment. In the L_α phase the two segments exhibit order parameters (C-6) of about 0·75 and (C-13) of about 0·35, respectively. As expected, conformational order decreases towards the end of the chains. At the main transition both order parameters jump to considerably higher values. Lowering the temperature increases conformational order at both positions. Note, however, that the terminal units order faster than the central ones. Thus, in the $L_{\beta'}$ phase below 250 K, the order parameter of both segments is greater than 0·9. For steric reasons, the C-2 segment of the *sn*-2-chain adopts unusual conformations, favouring gauche states. In the L_α phase ($T = 308$ K) the equilibrium populations, evaluated from different relaxation experiments, are $p_t = 0{\cdot}08$, $p_{g^+} = 0{\cdot}68$ and $p_{g^-} = 0{\cdot}24$, respectively. However, for the $L_{\beta'}$ phase at 273 K we obtain $p_{g^+} = 0{\cdot}9$ and $p_{g^-} = 0{\cdot}1$.

4. Discussion

The present knowledge about the molecular properties of phospholipid membranes originates to a large extent from N.M.R. studies using ^{1}H, ^{2}H, ^{13}C and ^{31}P nuclei [1–9]. Line shape investigations have been used to provide information about the dynamic organization of the bilayer systems. This approach is useful for examining orientational and conformational order in different parts of the membrane, and slow molecular motions in certain phases. For a complete molecular characterization, however, N.M.R. relaxation studies are required. The use of specifically deuteriated lipids offers the chance to detect the various molecular motions unambiguously. In addition, molecular order in the different phases can be determined.

The dynamics of DMPC membranes are specified in terms of five different correlation times, $\tau_{R\parallel}$, $\tau_{R\perp}$, τ_J(C-2), τ_J(C-6) and τ_J(C-13). They refer to overall rotation, overall fluctuation and trans–gauche isomerization of the chain segments, respectively. Arrhenius plots of the various correlation times are shown in figure 3.

In the L_α phase trans–gauche isomerization occurs with correlation times of $10^{-12}\,\text{s} < \tau_J < 5 \times 10^{-10}\,\text{s}$. Apparently, there is a mobility gradient along the chains. Note that τ_J decreases drastically from the C-2 to the C-13 segment. This result is in qualitative agreement with previous observations [8, 18–20] and theoretical predictions [21]. The activation energy E_J of 15 kJ/mol at C-6 and C-13 is slightly larger than the potential barrier height encountered in rotational isomerization about

C–C bonds [22]. A value for E_J of 45 kJ/mol, determined for C-2, corresponds to the unusual gauche–gauche isomerization process at this segment [17].

The correlation times for overall rotation and overall fluctuation range from 10^{-9} s to 10^{-7} s, exhibiting a constant anisotropy ratio $\tau_{R\perp}/\tau_{R\parallel}$ of 10. The change of the quadrupole echo line shape with the pulse separation clearly shows that molecular fluctuations are significant in this phase [4]. Motional activation energies of $E_{R\parallel} = E_{R\perp} = 60$ kJ/mol reflect the intermolecular character of these motions, representing reorientations of the lipid molecules as a whole.

Molecular fluctuations may occur as isolated or collective modes. For the latter mechanism, known as director fluctuations, a continuous distribution of correlation times is expected [23]. T_{1Z} measurements of phospholipid membranes at different Larmor frequencies in the MHz range have been interpreted in terms of the collective model [24]. This interpretation is at variance with recent proton T_1 dispersion measurements, carried out over a frequency range of six orders of magnitude (100 Hz $\leqq \omega_0/2\pi \leqq$ 300 MHz). The measurements show clearly that director fluctuations do not constitute a major relaxation mechanism in the MHz range [25]. Rather, isolated motions of individual molecules account fully for the observed T_1 data. Collective order fluctuations are observed only at extremely low frequencies in the kHz regime by a characteristic $T_1(\omega_0) \sim \omega_0^{-1}$ dispersion law, predicted for smectic type liquid crystals [25].

At the main transition all motions slow down abruptly and then decrease continuously upon further cooling. The activation energies for the two intermolecular processes $E_{R\parallel} = E_{R\perp} = 90$ kJ/mol correspond to those obtained above the main transition. The rate of trans–gauche isomerization at C-6 and C-13 slows down with a constant activation energy E_J of 8 kJ/mol. Again a mobility gradient for these motions can be detected, which, however, is less pronounced than in the L_α phase.

Throughout the $P_{\beta'}$ phase, the lipid bilayers exhibit a heterogeneous dynamic behaviour. A mobile component is detected, showing similar intermolecular mobility, as found in the L_α phase (overall rotation and fluctuation). The dynamics of the second component are restricted to small angular fluctuations, compatible with the observed chain packing in the $P_{\beta'}$ and $L_{\beta'}$ phases [26, 27].

Finally, we wish to discuss the conformational dynamics at the C-2 segment, which present a unique feature of the *sn*-2-chain. As proposed by Fuson and Prestegard [17], this motion consists of rotational jumps between two conformers having approximate gauche$^+$ and gauche$^-$ conformations about the C2–C3 bond. If we use the glycerol backbone as a frame of reference, the jumps between the two isomers introduce additional two-site jumps about the *sn*-2 chain axis, which affect all segments equally. Apparently, this motion dominates ^{2}H T_{1Z} relaxation, implying considerably faster conformational dynamics at C-6 and C-13 than previously assumed [8].

It should be noted that further N.M.R. investigations employing two-dimensional relaxation methods [28] are in progress. Fourier transformation with respect to the relaxation period τ_1 of a particular pulse sequence (see §2.2) yields two-dimensional N.M.R. spectra, which may be regarded as a graph of the relevant natural widths versus the resonance positions of the individual dynamic spin packets that constitute the spectrum [29]. For example, cross sections through the two-dimensional quadrupole echo spectrum along ω_1 provide homogeneous lineshapes associated with the spin–spin relaxation time T_{2E} [28, 30]. It is found that both the magnitude of T_{2E} and the way in which T_{2E} changes across the spectrum are very dependent upon the character of

the molecular motion responsible for spin relaxation [28, 29]. Thus two-dimensional relaxation spectroscopy offers a powerful means for unambiguous dynamic characterization of complex systems such as phospholipid membranes.

The molecular order of DMPC bilayers is specified by three different order parameters, S_{ZZ}, $S_{Z'Z'}$(C-6) and $S_{Z'Z'}$(C-13). They refer to the orientational order of the lipid molecule as a whole and the conformational order at the C-6 and C-13 segment, respectively. In figure 4 these order parameters are plotted as a function of temperature. Dashed lines indicate different phase transitions. In the L_α phase the orientational order parameters increase continuously with decreasing temperature. The values obtained ($0{\cdot}4 < S_{ZZ} < 0{\cdot}6$) agree closely with those obtained previously with *rigid* E.S.R. spin probes in oriented DMPC bilayers [31, 32]. Apparently, the rigid body order parameter S_{ZZ} is much less than unity in the L_α phase.

We now discuss the conformational order in this phase. At 318 K the segmental order parameter at C-6 $S_{Z'Z'}$ is 0·73, while the C-13 segment exhibits a considerably lower value $S_{Z'Z'}$ of 0·34. Apparently there is an order or flexibility gradient along the chains [33]. Interpretation of this order gradient in terms of statistical mechanical models of liquid crystals presents a challenging theoretical problem. Strictly, the deuterons of the various CD_2 groups are not completely equivalent. However, except for the 2-position, this inequivalence has not been detected [34, 35]. It appears that unequal gauche populations, as determined for C-2, are prerequisite to this observation [8, 35]. In general, however, these gauche conformations are equally populated, giving rise to a single quadrupolar splitting only.

The orientational order in the $P_{\beta'}$ phase is heterogeneous on a molecular level as two different components are observed [4, 8, 36]. Appropriate simulation of these components is only possible with equal conformational but different orientational order. Interestingly, the obtained order parameters S_{ZZ} of about 0·6 and 0·9 correspond to those observed in the L_α and $L_{\beta'}$ phases, respectively. A unique structural interpretation of this result is not yet possible. The fraction of the higher ordered component strongly increases with decreasing temperature. Thus, 10 K below the pretransition only the high order component is left. At 250 K the corresponding order parameter S_{ZZ} is 0·97, indicating a high degree of order and tight packing of the lipid molecules in the $L_{\beta'}$ phase [27, 37].

Finally, we discuss the conformational order in the low temperature phases. At the main transition the segmental order parameters jump from $S_{Z'Z'}$ of 0·77 to $S_{Z'Z'}$ of 0·95 and from $S_{Z'Z'}$ of 0·38 to $S_{Z'Z'}$ of 0·80. The abrupt increase of $S_{Z'Z'}$ reflects the strong reduction of the number of gauche conformers in the $P_{\beta'}$ phase [27]. Lowering the temperature increases conformational order at both positions. Note, however, that the terminal units order faster than the central ones. Interestingly, there is no discontinuity of $S_{Z'Z'}$ at the pretransition, in agreement with infrared studies, which shows that the gauche population does not change to a measureable extent on passing from the $P_{\beta'}$ to the $L_{\beta'}$ phase [27]. At temperatures less than 250 K the segmental order parameter is $S_{Z'Z'}$ greater than 0·9 for both positions, consistent with a fully extended all-trans chain [27, 37, 38].

References

[1] SEELIG, J., 1977, *Q. Rev. Biophys.*, **10**, 353.
[2] MANTSCH, H. H., SAITO, H., and SMITH, I. C. P., 1977, *Prog. N.M.R. Spectrosc.*, **11**, 211.
[3] GRIFFIN, R. G., 1981, *Methods Enzymol.*, **72**, 108.
[4] MEIER, P., OHMES, E., KOTHE, G., BLUME, A., WEIDNER, J., and EIBL, H. J., 1983, *J. phys. Chem.*, **87**, 4904.

[5] DAVIS, J. H., 1983, *Biochim. biophys. Acta*, **737**, 117.
[6] SMITH, R. L., and OLDFIELD, E., 1984, *Science, N.Y.*, **225**, 280.
[7] BLOOM, M., and SMITH, I. C. P., 1985, *Progress in Protein–Lipid Interactions*, edited by A. Watts and DePont (Elsevier), p. 61.
[8] MEIER, P., OHMES, E., and KOTHE, G., 1986, *J. chem. Phys.*, **85**, 3598.
[9] DAVIS, J. H., 1986, *Chem. Phys. Lipids*, **40**, 223.
[10] DAS GUPTA, S. K., RICE, D. M., and GRIFFIN, R. G., 1982, *J. Lipid Res.*, **23**, 197.
[11] FINEGOLD, L., and SINGER, M. A., 1986, *Biochim. biophys. Acta*, **855**, 417.
[12] MÜLLER, K., MEIER, P., and KOTHE, G., 1985, *Progr. N.M.R. Spectrosc.*, **17**, 211.
[13] MORO, G., and FREED, J. H., 1981, *J. chem. Phys.*, **74**, 3757.
[14] REDFIELD, A. G., 1965, *Adv. magn. Reson.*, **1**, 1.
[15] TORCHIA, D. A., and SZABO, A., 1982, *J. magn. Reson.*, **49**, 107.
[16] LIPARI, G., and SZABO, A., 1982, *J. Am. chem. Soc.*, **104**, 4546.
[17] FUSON, M. M., and PRESTEGARD, J. H., 1983, *Biochemistry*, **22**, 1311.
[18] BROWN, M. F., SEELIG, J., and HÄBERLEN, U., 1979, *J. chem. Phys.*, **70**, 5045.
[19] JEFFREY, K. R., WONG, T. C., and TULLOCH, A. P., 1984, *Molec. Phys.*, **52**, 307.
[20] MOSER, M., MARSH, D., MEIER, P., WASSMER, K. H., and KOTHE, G., *Biochemistry* (submitted).
[21] FERRARINI, A., MORO, G., and NORDIO, P. L., 1988, *J. chem. Phys.* (in the press).
[22] FLORY, P. J., 1969, *Statistical Mechanics of Chain Molecules* (Interscience).
[23] WADE, C. H., 1977, *A. Rev. phys. Chem.*, **28**, 47.
[24] BROWN, M. F., 1982, *J. chem. Phys.*, **77**, 1576.
[25] ROMMEL, E., NOACK, F., MEIER, P., and KOTHE, G., *J. phys. Chem.* (in the press).
[26] JANIAK, M. J., SMALL, D. M., and SHIPLEY, G. G., 1979, *J. biol. Chem.*, **254**, 6068.
[27] CAMERON, D. G., CASAL, H. L., MANTSCH, H. H., BOULANGER, Y., and SMITH, I. C. P., 1981, *Biophys. J.*, **35**, 1.
[28] MÜLLER, K., SCHLEICHER, A., and KOTHE, G., 1988, *Molec. Crystals liq. Crystals.* (in the press).
[29] MILLHAUSER, G. L., and FREED, J. H., 1984, *J. chem. Phys.*, **81**, 37.
[30] MÜLLER, L., and CHAN, S. I., 1983, *J. chem. Phys.*, **78**, 4341.
[31] MEIER, P., BLUME, A., OHMES, E., NEUGEBAUER, F. A., and KOTHE, G., 1982, *Biochemistry*, **21**, 526.
[32] LANGE, A., MARSH, D., WASSMER, K.-H., MEIER, P., and KOTHE, G., 1985, *Biochemistry*, **24**, 4383.
[33] SEELIG, A., and SEELIG, J., 1974, *Biochemistry*, **13**, 4839.
[34] SEELIG, J., and SEELIG, A., 1980, *Q. Rev. Biophys.*, **13**, 19.
[35] ENGEL, A. K., and COWBURN, D., 1981, *FEBS Lett.*, **126**, 169.
[36] WITTEBORT, R. J., SCHMIDT, C. F., and GRIFFIN, R. G., 1981, *Biochemistry*, **20**, 4223.
[37] HAUSER, H., PASCHER, I., PEARSON, R. H., and SUNDELL, S., 1981, *Biochim. biophys. Acta*, **650**, 21.
[38] CAMERON, D. G., CASAL, H. L., GUDGIN, E. F., and MANTSCH, H. H., 1980, *Biochim. Biophys. Acta*, **596**, 463.