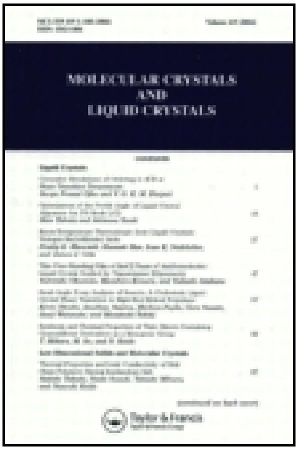

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Headgroup Mobility in the Low Temperature Phase (Lσ′) of DPPC

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Headgroup Mobility in the Low Temperature Phase (Lσ′) of DPPC

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The ^{1}H decoupled Phosphorus NMR lineshape of DPPC–water dispersions shows characteristic changes when passing through the three thermotropic phase transitions: subtransition (Lσ′ – Lβ′), pretransition (Lβ′ – Pβ′) and main transition (Pβ′ – Lα). At the subtransition the spectrum changes from a non-axial-symmetric powder spectrum (Lσ′) to an axial-symmetric one (Lβ′). The two higher phase transitions are characterized by decreases in the intrinsic ^{31}P line widths and the chemical shift anisotropy.

Proton-enhanced ^{13}C-spectra of the same compound, with natural isotope abundance, recorded at 76 MHz permit the resolved observation of different carbon-positions in the headgroup. In particular the N–$(CH_3)_3$–carbon nuclei exhibit a narrow isotropic lineshape throughout the entire observed temperature range (−20°C . . . 50°C). This implies that rotational motion in this moiety does not cease below the subtransition.

To summarize, the headgroup mobility in the Lσ′-phase can be described as follows: The rotation of the entire choline group about the chemical bonds between the phosphate group and the glycerol-backbone is slow on the ^{31}P-NMR timescale, the N–$(CH_3)_3$ end of the choline moiety, however, still undergoes rapid rotational motion. This picture is in accordance with recent interpretations of our ^{1}H-NMR second moment analysis.

INTRODUCTION

The headgroup structure of phospholipids organized in planar bilayers has been investigated for several years by a variety of methods, such as neutron diffraction[1] and nuclear magnetic resonance (NMR).[2] In particular ^{31}P–NMR studies supported the view that the headgroup,

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carrying the electric dipole, is aligned parallel to the bilayer plane.[3] From the axial symmetry of the spectra it was further concluded that both, above and below the main transition temperature T_c, this moiety undergoes rapid rotational motion.[4-8] This mobility was observed to cease only at very low temperatures[9] or upon reduction of the water content.[10] In addition, it was assumed that disordered fluctuations of the molecule are present, leading to a further reduction of the chemical shift anistropy of the ^{31}P-chemical shift tensor.

The most detailed studies of this kind were carried out on bilayers of fully hydrated dipalmitoylphosphatidylcholine (DPPC). This particular model system exhibits four phases in the temperature range −30°C to 80°C, here referred to as $L\sigma'$, $L\beta'$, $P\beta'$, and $L\alpha$.[10] Since the low temperature phase $L\sigma'$ was not known to exist until 1980,[12] the headgroup studies mentioned above dealt with the structures in the latter three phases only. Since then ^{31}P–NMR studies of Füldner[13] and Marsh,[14] and ^{1}H–NMR results from our laboratory[15] have given some support to the idea that the headgroup motion in the $L\sigma'$-phase is considerably restricted. Phosphorus spectra below the "subtransition" from $L\sigma'$ to $L\beta'$ showed a deviation from an axial symmetric lineshape. In the case of ^{1}H–NMR a separate monitoring of the fast moving headgroup protons and the less mobile remainder of the molecule becomes possible by a second moment analysis.[16] Interestingly, in the $L\sigma'$-phase the relative contribution of the mobile protons to the observed signal was found to be only 11% compared to 16% in $L\beta'$, which is equivalent to a freeze of ~ 4 protons per molecule.[15] This led us to propose that in the low temperature phase $L\sigma'$ the rotation of the entire choline group ceases, whereas fast motions of the 9 terminal protons about the a_6 and a_7 bonds (see Figure 1) are still present and give rise to the residual 11% signal contribution of rapidly moving protons.

It is the purpose of the present work to test this view by further experiments, which are intended to give more detailed information about the headgroup structure. To obtain insight into the dynamics at the terminal methyl groups of the choline moiety, we have chosen ^{13}C–NMR in natural abundance, a method, that permits a separate observation of the N–$(CH_3)_3$–carbons.[17] An extensive ^{31}P–NMR study was applied to monitor the motional behaviour at the phosphate position, where the headgroup is linked to the nonpolar part of the molecule. The results strongly support the suggested model and in addition, give an instructive illustration of the kinetics of the temporal transition from $L\beta'$ to $L\sigma'$.

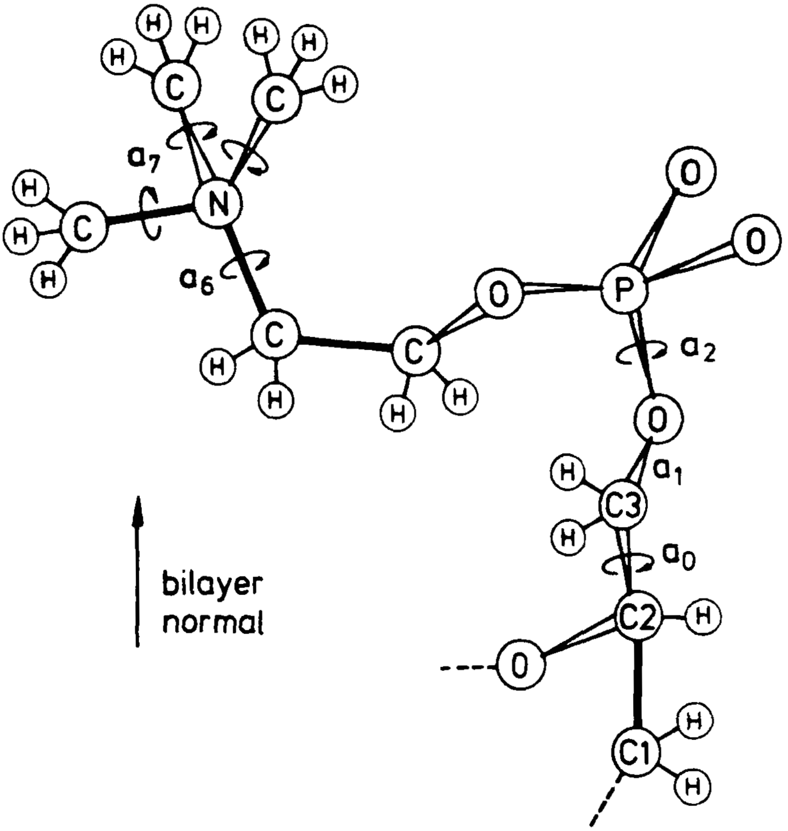

FIGURE 1 A schematic sketch of the headgroup structure of DPPC. Bonds, which represent possible axes of rotations, are indicated by a_0, a_2, a_6, a_7.

MATERIALS AND METHODS

DPPC was purchased from Sigma Chemie GmbH, Munich, FRG. Thin layer chromatrography of this compound exhibited a single spot, and no purification procedures were applied. 40% water was added following the cold swelling procedure described elsewhere.[17,18] Finally the sample was sealed in a glass tube under a nitrogen atmosphere. Before recording spectra of the Lσ'-phase, the sample was kept at 0°C for at least 48 h, unless indiated otherwise.

The measurements were performed on a Bruker CXP-300 NMR-spectrometer with ^{13}C and ^{31}P resonance frequencies of 76 and 121 MHz, respectively. A ^{1}H decoupling field of 300 MHz was irradiated during the acquisition time. For ^{31}P-measurements this rf-power was kept as low as possible to avoid sample heating. In case of ^{13}C–NMR the signal to noise ratio was improved by polarization transfer from the protons (PE–NMR),[19] where the ^{1}H and ^{13}C rf-power levels have to match the Hartmann-Hahn condition.[20]

THEORY

If decoupled from the dipolar interaction with the protons, both ^{13}C and ^{31}P-spectra, are governed by the chemical shift. As outlined in the monograph of Haeberlen,[21] the chemical shift, observed in an NMR-experiment, can be described by a symmetric 3-dimensional second rank tensor characterized by three principle components, σ_{11}, σ_{22}, and σ_{33}. In presence of fast rotational motion about a fixed axis, the static tensor is averaged to an axial symmetric one, where two of the principal values become equal. The anisotropy of the static chemical shift tensor

$$\Delta\sigma_{\text{stat}} = \sigma_{33} - \frac{1}{2}(\sigma_{11} + \sigma_{22}) \tag{1}$$

is reduced to

$$\langle\Delta\sigma\rangle_{\text{rot}} = \sigma_{\parallel} - \sigma_{\perp}, \tag{2}$$

where $\sigma_{\parallel}$ and $\sigma_{\perp}$ refer to the nondegenerate, and the degenerate principal values of the averaged tensor, respectively. The reduction factor is given by

$$R_{\text{rot}} = \frac{\langle\Delta\sigma\rangle_{\text{rot}}}{\Delta\sigma_{\text{stat}}} = \frac{1}{2}(3\cos^2\theta - 1 - \eta\sin^2\theta\cos2\phi), \tag{3}$$

where θ and Φ are polar and azimuthal coordinates of the rotation axis in the principal axis system of the static tensor.[22] η describes the asymmetry of the static tensor

$$\eta = \frac{\sigma_{22} - \sigma_{11}}{\sigma_{33} - \sigma_{\text{iso}}}. \tag{4}$$

σ_{iso} is the average value of the principal components, which can be directly observed in liquids, where rapid isotropic motions lead to a complete averaging of the chemical shift.

The anisotropy of fluctuations is usually expressed in terms of the order parameter

$$S = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle, \tag{5}$$

where θ describes the time dependent deviation from the average orientation, and the arrowhead brackets indicate a temporal average.[23] In absence of motion, $S = 1$, whereas isotropic tumbling leads to $S = 0$. For an axial symmetric tensor the reduction of $\Delta\sigma$ by this type of motion is directly given by

$$R_{\text{fluct}} = \frac{\langle\Delta\sigma\rangle_{\text{fluct}}}{\Delta\sigma} = S \tag{6}$$

If the motions are not considerably faster than the NMR-time-scale, the averaging is incomplete. The shape of the resulting slow motion spectra is discussed in detail in the monographs of Mehring[24] and Spiess.[25] An application to the particular case of ^{31}P–NMR of phospholipids was given by Campbell et al.[9]

RESULTS AND DISCUSSION

Proton enhanced ^{13}C–NMR spectroscopy is known to be a powerful technique for the study of molecular dynamics.[19] In particular, for the investigation of phase transitions in phospholipid bilayers, this method has been applied frequently in recent years to both natural and isotopically labelled compounds.[17,26–28] The spectra presented in Figure 2 were recorded in DPPC of natural ^{13}C-abundance at four temperatures, each one representative of one of the four phases Lσ', Lβ', Pβ', and Lα. The decoupling of the dipolar interaction with the protons provides a spectral resolution, which allows a separation of the signal of the hydrocarbon chains $(CH_2)_n$, their terminal methyl groups $(CH_3)_2$, and the headgroup. It is interesting in this context that the resonance line of the three N–$(CH_3)_3$– carbons is clearly resolved. Its width remains unchanged at ~ 5 ppm throughout the whole observed temperature range (−10°C to 50°C). Evidently, the motional changes occurring at the "subtransition" between Lσ' and Lβ' do not cause further averaging of the chemical shift tensor of these nuclei.

This observation is easily understood in terms of the proposed model. Superimposed rotations of the carbon nuclei about the a_7 and

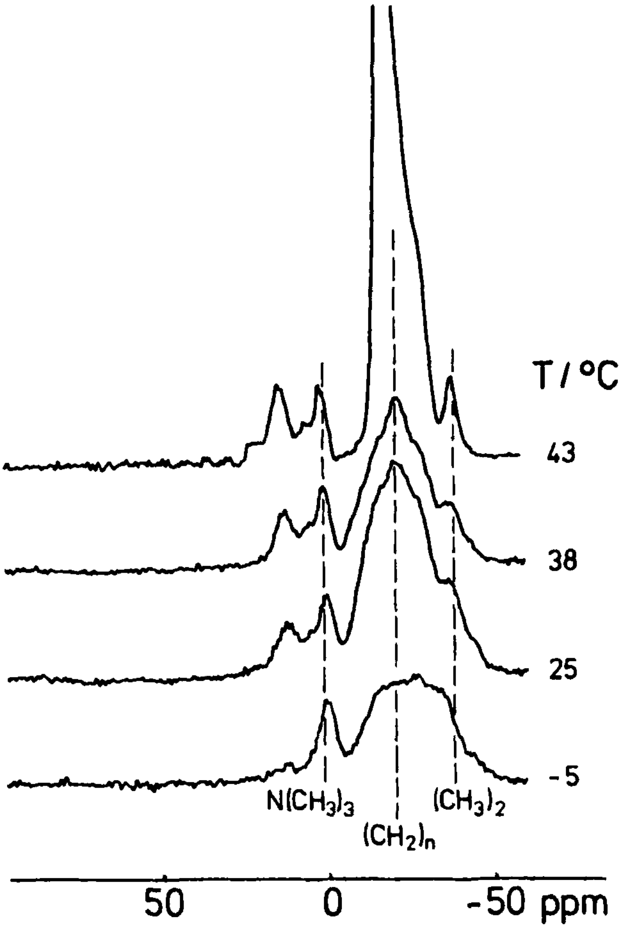

FIGURE 2 Proton enhanced and decoupled ^{13}C spectra recorded in the four phases of DPPC. The origin of the ppm-scale is chosen arbitrarily.

a_6 bonds (see Figure 1) are sufficient to cause a collapse of the chemical shift anisotropy of the methyl carbons. The resulting line is isotropic-like and cannot be reduced any further by the onset of a third rotation of the choline moiety as a whole. Thus, these results confirm the view, that the rapid motion in the N–$(CH_3)_3$–group does not freeze in the Lσ'-phase. The observed parameter is, however, insensitive to the presence of further motions, and, consequently, no implications concerning the motion of the whole choline moiety can be drawn. This information is provided by investigation of the ^{31}P magnetic resonance. The temperature dependence of the spectra is illustrated in Figure 3. Since the sample consists of randomly orientated bilayers, the well known powder shape due to the anisotropy of the ^{31}P-chemical shift is observed, where the positions of edges and maximum of the spectral distribution reflect the principal values of the chemical shift tensor. All of the three known phase transitions of DPPC, occurring with increasing temperature, are indicated by discontinuous changes of the spectral patterns. The most striking effect—and most interesting in our context—is evident at the "subtransition" between Lσ' and Lβ' at 12°C.

Below this temperature the spectra indicate the presence of three principal values of the chemical shift tensor. This feature is most clearly pronounced at even lower temperatures (−20°C), whereas immediately below the subtransition, the edges are blurred by slow motional averaging (see also refs. 9, 13 and 14). The principal values of these spectra measured at −20°C referred to the isotropic value are σ_{33} = 107 ppm, σ_{22} = −26 ppm, σ_{11} = −81 ppm, which is in good agreement with the static chemical shift tensor of the monohydrate of DPPC.[8] Consequently, the structure of fully hydrated DPPC bilayers at the phosphorus position at low temperatures of the Lσ' phase can be considered as rigid on the timescale of the ^{31}P-NMR measurements ($\sim 10^{-5}$ s). At the highest temperatures of the Lσ'-phase motions with correlation times comparable with this timescale are present, leading to the slow motional spectra around 10°C.

Above this temperature the spectra are totally different: They become axially symmetric (η = 0) and their anisotropy changes in absolute value and sign to $\Delta\sigma$ = −65 ppm (14°C). This behavior is the direct corroboration of the proposed model. In Lσ' the motion of the choline group as a whole, is restricted—leading to rigid lattice spectra at the phosphorus position, which become insufficiently averaged only towards higher temperatures. In the other three phases, which have been the subject of most of the ^{31}P-studies on this system so far, a rotation of the entire choline group about one of the chemical bonds which connect it to the nonpolar part of the molecule, such as a_2, a_0, is present (see Figure 1). From the observed changes of $\Delta\sigma$, an unambiguous determination of the bond of rotation is not possible. According to the crystallographic data concerning the headgroup structure and the orientation of the ^{31}P chemical shift tensor in the molecular frame, neither a_2 nor a_0 can be excluded as possible rotation axes. However, we wish to emphasize here that our previous ^{1}H-NMR observations support the view that a_2 is the bond of rotation. Otherwise the rotational motion would effect also the two methylene protons at the C3 position. Consequently, we would expect, that at

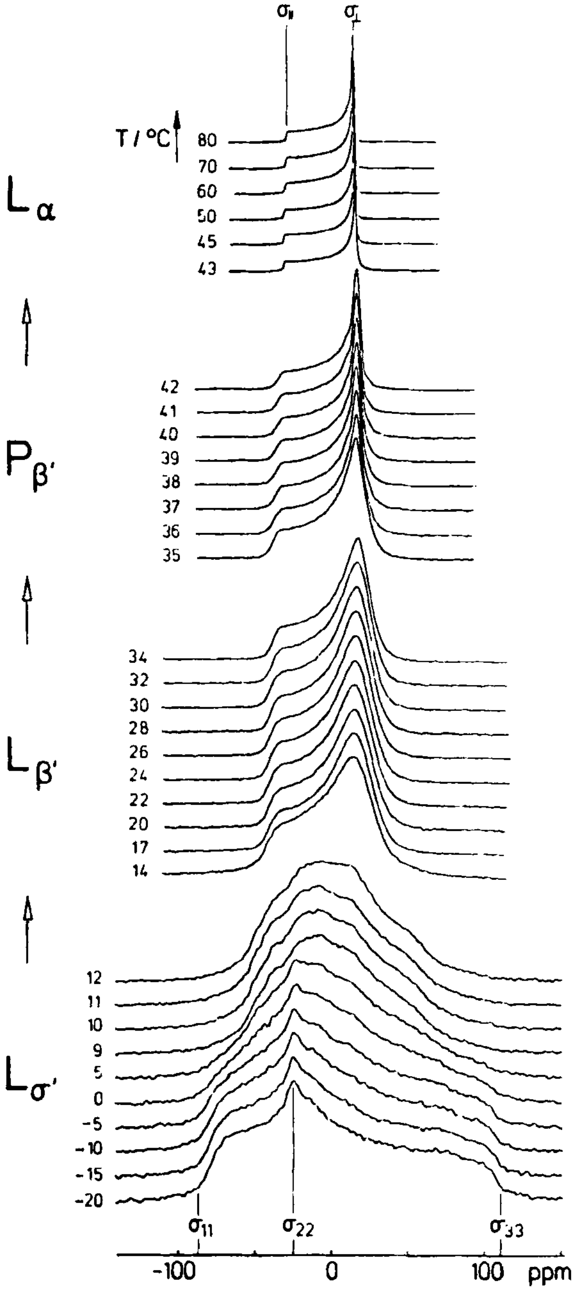

FIGURE 3 Temperature dependence of proton decoupled ^{31}P–NMR spectra. The ppm-scale is referred to the isotropic value of the chemical shift tensor. The stacked plots are divided into four groups, representing the four phases of DPPC Lσ', Lβ', Pβ' and Lα.

the subtransition the number of rapidly moving protons would change from 9 to 15—in contradiction to the observed change from 9 to 13.

Another interesting phenomenon of the Lσ'-phase is its slow temporal development, after cooling from room temperature to 0°C. Immediately after cooling, the sample remains in the Lβ' phase, indicated by the axial symmetry of the ^{31}P-spectra. With $\Delta\sigma = -69$ ppm the chemical shift anisotropy has reached its extreme value; cooling to even lower temperatures (-10°C) does not result in further spectral changes. The temporal transition from Lβ' to Lσ' is illustrated in Figure 4. The axially symmetric powder pattern gradually changes into the asymmetric type. After ~ 50 h the transition is complete, and no more changes in the spectral shape are observed. Note, that the imtermediate spectra are not superpositions of the Lβ' and Lσ' spectra but instead resemble those observed for the Lσ' phase shortly below the subtransition. This temporal transition cannot, therefore, be visualized as a growth of crystalline domains of Lσ' regions. It appears more appropriate to consider this process as a gradual slowing down of headgroup mobility, which takes place simultaneously over the whole sample.

Finally, we wish to discuss the temperature dependence of the ^{31}P-spectra in the three other phases Lβ', Pβ', and Lα. The variation of

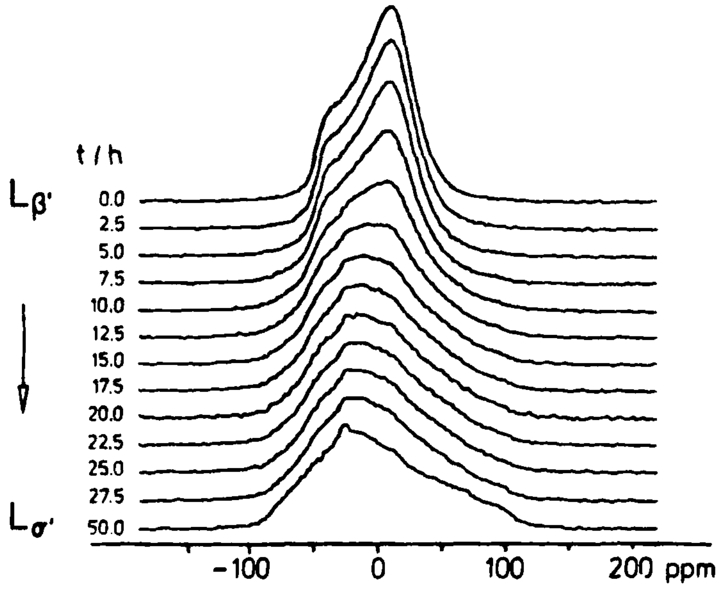

FIGURE 4 Temporal development of the proton decoupled ^{31}P–NMR spectrum of DPPC stored at 0°C.

the chemical shift anisotropy with temperature is plotted in Figure 5. The data below 12°C were recorded after cooling down from room temperature. As mentioned above, this procedure yields axial symmetric spectra down to −10°C. Note also the plateau in $\Delta\sigma(T)$ reached below 0°C. At higher temperatures the Lβ′-phase is characterized by a continuous decrease of $\Delta\sigma$ with increasing temperature. Altogether, the range of $\Delta\sigma$ in the Lβ′-phase is from −69 to ~ −60 ppm. In accordance with earlier workers[2–4] we attribute this behavior to an increase in the random fluctuations of the molecule, which can be characterized by an order parameter $S < 1$. It is evident from Figure 3 that the intrinsic line width of the ^{31}P resonance also decreases slightly towards higher temperatures of the Lβ′-phase. One may again attribute this to the increasing random motion.

In contrast to earlier ^{31}P–NMR work, the resolution of these measurement permits the observation of pronounced effects at the pretransition. In the range around 34°C $\Delta\sigma$ drops by ~ 8% to −56 ppm. More drastically, the average intrinsic line width narrows by a factor of ~ 2. A detailed discussion of the complex structure of the Pβ′-phase is outside the scope of this article, but we wish to mention that

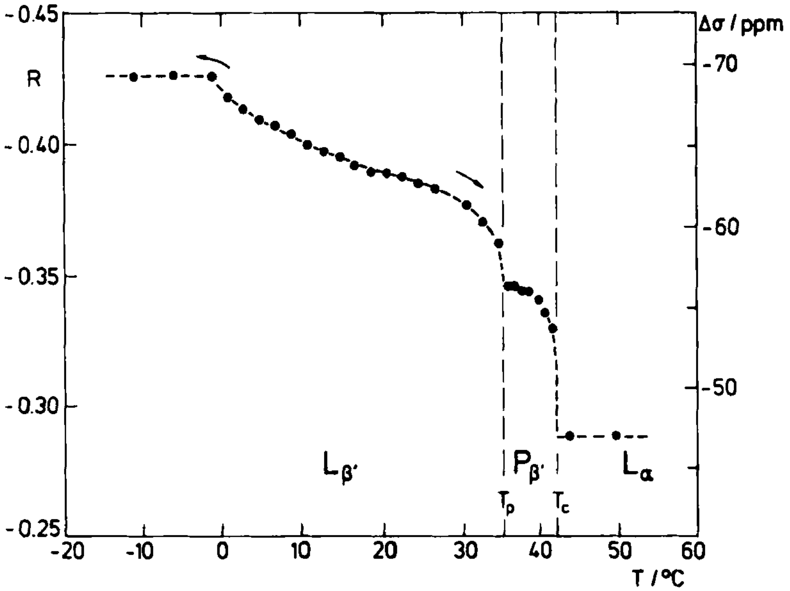

FIGURE 5 Temperature dependence of the chemical shift anisotropy in 3 phases Lβ′, Pβ′ and Lα.

these findings are in agreement with NMR-observations of other nuclei, such as ^{13}C [15] and ^{1}H.[15,29] However, in contrast to several other NMR studies,[26,27,30,31] we do not observe a superposition of Lβ' and Lα structures in the Pβ'-phase. This inhomogeneity is seen in connection with distortions, that may be caused by periodic bilayer undulations ("ripples"), which are known to occur in the Pβ'-phase. We wish to leave this point and deal with it in a future publication.[32]

At the main transition the chemical shift anisotropy again drops discontinuously by ~ 20%. The residual line width, in turn, narrows far more dramatically. This latter effect can easily be explained in terms of the well known drastic dynamical changes at this phase transition. The relatively slight effect in $\Delta\sigma$ can also be understood, if one considers the origin of the axial symmetry of the spectra. The Lα-phase is characterized by fast lateral diffusion of the lipid molecules in the plane of the bilayer. This anisotropic motion is accompanied by fast rotational diffusion about the long molecular axis. If the orientation of this axis forms only a small angle with the axis of intramolecular rotation, present also at lower temperatures (say a_2, the P–O bond), the changes in $\Delta\sigma$ will be only small. A detailed discussion of this interesting problem of superpositions of rotational diffusions will be given in a future publication.[33] In this context it is important to note that the geometry of motion is reflected in the chemical shift anisotropy, whereas the rate determines the intrinsic line width.

CONCLUSION

A model of the headgroup motion in the Lσ'-phase of DPPC bilayers, originally suggested on the basis of simple ^{1}H–NMR measurements, is strongly supported by the results of the present work. This evidence is drawn from the well resolved ^{13}C-resonance of the terminal methyl groups of the choline moiety, and a detailed analysis of the ^{31}P-resonance. According to this view, the fast overall intramolecular rotation of the choline group about the rest of the molecule, which is known to be present in the other three phases, Lβ', Pβ', and Lα, is considerably restricted in the Lσ'-phase. On the other hand, the fast motions in the terminal $N\text{–}(CH_3)_3$ regions are not affected by this phase transition. In addition, the data presented in this article provide an interesting insight into kinetics of the slow transition Lβ' ↔ Lσ'. Evidently this process is not governed by a gradual growth of domains of the Lσ′-phase, but rather by a collective slowing down of the choline rotation.

Low temperature phases are known to exist also in bilayers of related compounds, i.e. dipalmitoyl-, dimiristoyl-, and dilaureoyl-phosphatidylethanolamine.[34–37] It will be interesting to investigate whether the corresponding phase transitions are characterized by similar processes.

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