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NMR and X-Ray Investigation of the Phase Behavior of Phosphatidylethanolamines†

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The second moments of the proton magnetic resonance lines of DPPE and DMPE, below the main phase transition, vary within a similar range as those of the corresponding phosphatidylcholines. In contrast to the sharp "subtransition" between $L\sigma'$ and $L\beta'$ phase, observed in the latter class of lipids, both DMPE and DPPE exhibit a broad transition (~15 K width) to a less mobile state at lower temperatures ~ −20°C. The second moment of the hydrocarbon chain resonance line in this state is between the value observed in the $L\sigma'$ phase of DPPC and the higher value of the rigid lattice. In addition to these phenomena a time-dependent transition from the gel phase to a rigid crystalline state is observed in DMPE, with rigid lattice values for the second moments. This behaviour is presumably analogous to that found for DLPE.

The X-ray diffraction patterns indicate that a hexagonal lattice exists for the hydrocarbon chains throughout the entire observed temperature range below the main transition, with increasing density of chain packing towards lower temperatures. In the low temperature state, the corresponding reflection is appreciably broadened, due to the reduced long-range order. In addition, the lamellar spacing is drastically reduced (~10%) resulting from the loss of interlamellar water. The hydrocarbon chains are shown to remain untilted.

INTRODUCTION

Since the discovery of a low temperature phase in fully hydrated lecithins by Chen *et al.*,[1] the study of these ordered bilayer conformations has aroused considerable interest. In addition to differential

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scanning calorimetry several other spectroscopic methods have been employed, such as infrared absorption,[2,3] X-ray crystallography,[4-7] nuclear magnetic resonance (NMR),[8,9] and dilatometry.[10] At present it can be considered fairly certain that the packing of the hydrocarbon chains in this low temperature phase is similar to that of a paraffin lattice. This close packing leads to considerable restrictions in the mobility of the chains, evident from both infrared and NMR. In the preceding paper we demonstrated that these features are accompanied by a freezing of the choline headgroup rotation about the rest of the molecule.[11]

In addition to these observations on phosphocholines, similar phases have been observed in related compounds with two different chain lengths, i.e. dilaureoyl- and dimyristoyl-phosphatidylethanolamines (DLPE and DMPE).[12,13] In contrast to the former compounds, the gel phase of ethanolamines ($L\beta$) is characterized by the absence of a chain tilt with respect to the bilayer normal. This is possibly related to the absence of a rippled intermediate phase at temperatures below the main transition into the liquid crystalline phase ($L\alpha$). However, it has been found recently that the gel phase of both, DLPE and DMPE, is metastable over a wide temperature range.[14] Below 25°C a temporal transition takes place to a less hydrated state, referred to as "subgel" in the article of Wilkinson and Nagle. Since this state exhibited significantly less mobility, it appeared obvious to compare it with the low temperature phase of phosphocholines (referred to as $L\sigma'$ in our articles). However, in contrast to the latter crystallographic state, only a few structural and dynamical parameters of the "subgel" phase have been reported so far.

This article presents some new data on this ordered state—in particular, crystallographic data obtained by X-ray wide angle diffraction. We also report another motionally restricted state of DMPE and DPPE occurring at low temperatures. This fourth state of ethanolamine bilayers is markedly different from the "subgel" phase, and, consequently, it may become questionable which of the two phases, if any, is equivalent to the $L\sigma'$ phase of phosphocholines.

MATERIALS AND METHODS

DMPE and DPPE were used as purchased from Fluka GmbH, Neu-Ulm, and Sigma GmbH, Munich, without further purification. 50 wt.% water was added under nitrogen applying the cold swelling procedure described elsewhere.[15,16] Measurements concerning the gel

and the low temperature phase were recorded after heating the sample well above the main transition temperature for some 30 minutes. The temporal transformation into the subgel phase was observed at room temperature.

^{1}H-NMR spectra were recorded with a Bruker SXP-spectrometer operating at 80 MHz. Data storage and processing was done by a Hewlett-Packard MX-computer with homemade software.

For X-ray experiments the fully hydrated preparation of unoriented bilayers was done by a 50 wt.% lipid/water mixture in a Mark capillary. Another sample was oriented by a "multi-sandwich" of this mixture and mylar, as described earlier.[17] A water reservoir inside the capillary guaranteed full hydration at all temperatures.

THEORY

(Second moment analysis)

Strong dipolar broadening of the ^{1}H-NMR lines can conveniently be expressed in terms of their second moments (M_2). The correlation of this parameter with the dipolar interaction between the observed nuclei is given by

$$M_2 = \frac{9}{16}\gamma^4\hbar^2\sum_i\left\langle\frac{1-3\cos^2\theta_{ij}}{r_{ij}^3}\right\rangle^2 \qquad (1)$$

where r_{ij} is the distance between two interacting protons, θ_{ij} its angle with respect to the external magnetic field, γ is the gyromagnetic ratio, and $\hbar$ Planck's constant.[18] The angle brackets indicate the averaging of the geometrical term over the observation time. This expression describes the sum of interactions of one particular nucleus j with all of its neighbors. Evidently eq. (1) applies only if this proton is representative for all nuclei of the sample. In large, structured molecules, such as phospholipids, this cannot hold in general. Now, one can assume that most of the structural differences are averaged by spin diffusion. The most distinguished molecular regions, however, i.e. the mobile polar headgroup and the relatively less mobile chains, are separated by the phosphate and glycerol moiety with low proton densities. These regions represent barriers for spin diffusion, and consequently, a separate observation of headgroup and hydrocarbon chains is possible.[19] The M_2 analysis of this article will basically refer to the homogeneous hydrocarbon chain part of the ^{1}H-NMR signal.

RESULTS AND DISCUSSION

The variations of the ^{1}H-NMR second moment (M_2) of the hydrocarbon chains of DMPE and DPPE with temperature are shown in Figures 1a and 1b, respectively. In both plots the gel phase from ~0°C to the main transition temperature T_c at 49°C (DMPE) or 61°C (DPPE) is characterized by a steady decrease in M_2, with a constant slope until ~5 K below T_c. Except for the different main transition temperatures, the two homologous compounds exhibit identical absolute values in this range. Altogether, the second moments of the gel phase of both enthanolamines vary between 2.0 and 4.0 × 10^9 s^{-2}. Both, slope and absolute values of the second moments of this part of the plot are close to those found for the related compound

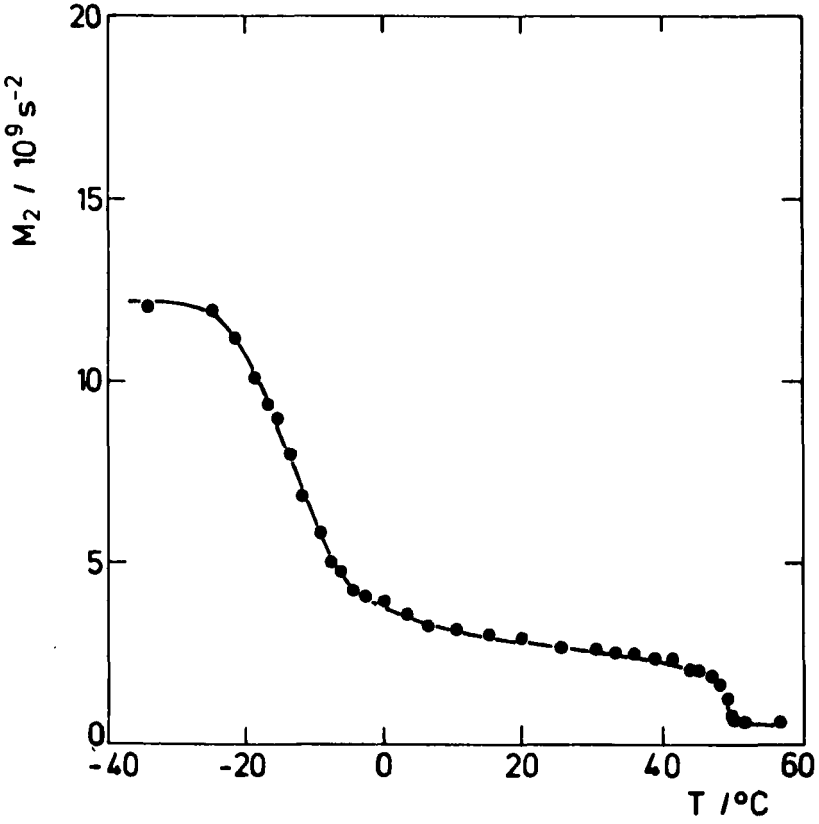

FIGURE 1a Development of the ^{1}H-NMR second moment *vs.* temperature evaluated for the hydrocarbon chains of DMPE

dipalmitoylphosphatidylcholine.[9,20] Unlike the results on phosphocholines, however, there is no step below the main transition temperature, indicating the absence of a pretransition in ethanolamines. Instead, there is a decrease in the slope ~5 K before passing the main transition. This result may be seen in accordance with recent ^{2}H-NMR investigations of Marsh *et al.*,[21] who observed a coexistence of Lβ and Lα phases several degrees below the main transition of DMPE. The enlargement of the main transition regions in Figure 2 illustrates another interesting phenomenon of the two homologous compounds. This phase transition is shown by a decrease in the second moment by a factor of ~4, an effect, which is easily understood as a result of the increased chain mobility in the liquid crystalline state.[22] Surprisingly, this transition with width ~5 K is about ten times broader

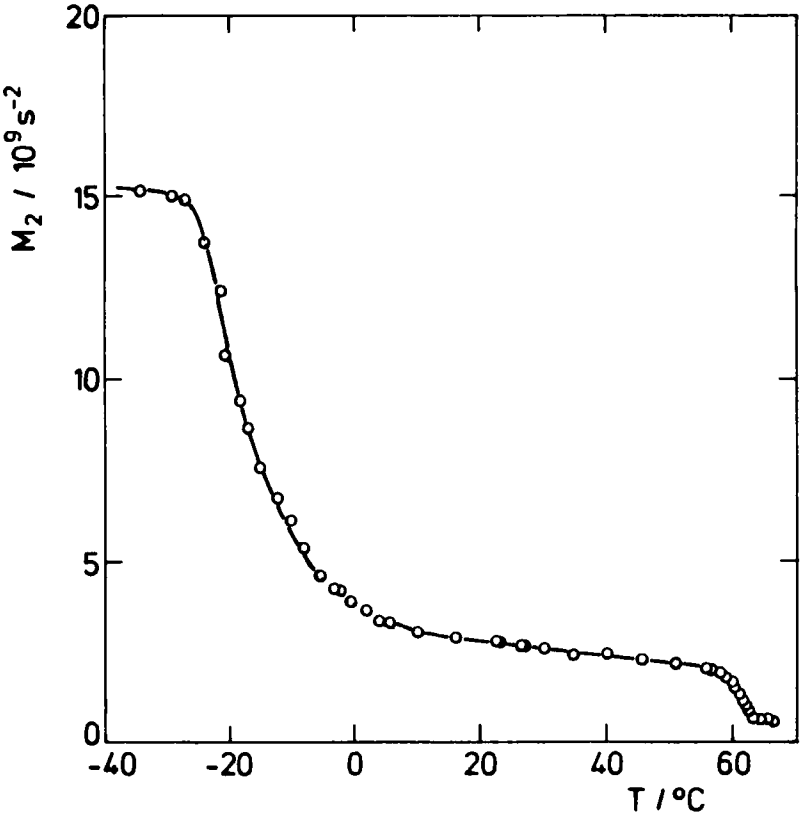

FIGURE 1b Development of the ^{1}H-NMR second moment for DPPE.

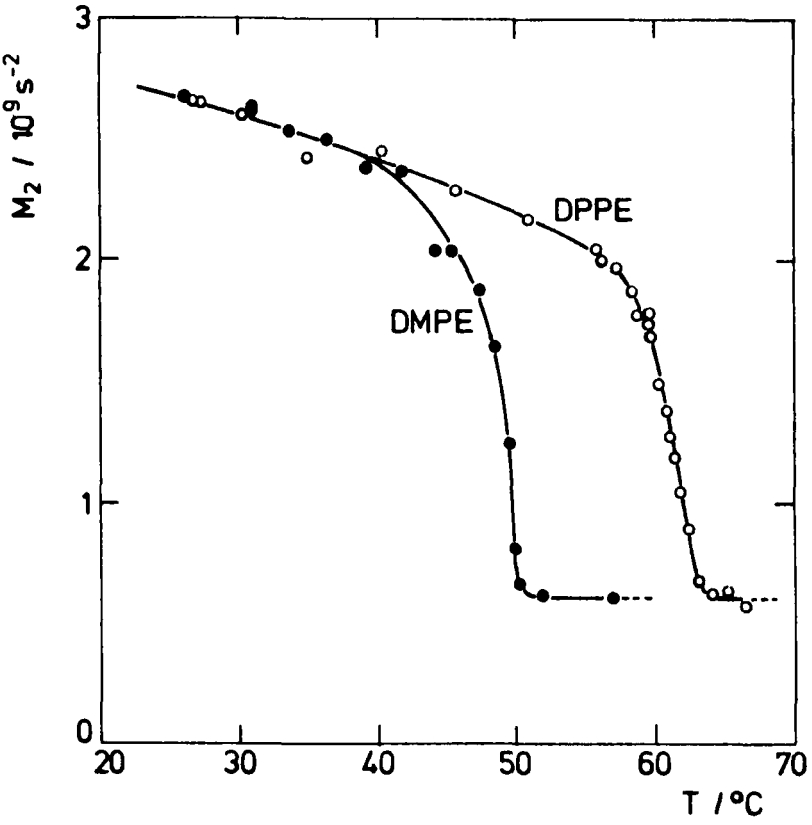

FIGURE 2 Enlargement of the main transition regions of Figure 1.

than its analogue in phosphocholines.[9,20] In summary, on the basis of our data, the gel-to-liquid crystalline-transition in ethanolamines can be visualized as follows: Even though there is no particular intermediate phase, like the Pβ′ state of the corresponding phosphocholines, there are distinct pretransitional effects, some 5 K below the main transition. In addition, this transition is significantly broadened.

Another interesting feature of the ^{1}H-NMR results is the behaviour below 0°C. In the range between 0°C and ~ −25°C the second moments of both DMPE and DPPE, increase considerably with decreasing temperature. Below −25°C the temperature dependence has reached a plateau, which is different for the two homologous compounds. For DMPE the final value of $M_2 = 12 \times 10^9$ s^{-2} is significantly lower than the rigid lattice value for hydrocarbon chains (~17 $\times 10^9$ s^{-2}),[9] whereas the plateau value of DPPE (~15 $\times 10^9$ s^{-2})

comes closer to this limit. In both cases the low temperature states of these compounds must be considered as substantially restricted in their motional freedom. We have also observed a drop in the relative contribution of the fast moving headgroup protons similar to that for the low temperature phase, $L\sigma'$ of phosphocholines, indicating a freeze of the overall headgroup rotation in the low temperature state of enthanolamines. In other respects the phase behavior of these compounds at low temperatures is markedly different from that of phosphocholines. We have not observed any temporal effects of this transition, and no hysteresis, which would indicate metastability. Note the extreme breadth of this transition—in contrast to the sharp subtransition of DPPC. Also, the final plateau values of M_2 are slightly higher than that observed in the $L\sigma'$ phase of DPPC. Further information concerning the molecular dynamics can be inferred from the spin lattice relaxation time (T_1) measurements (see Figure 3). Again, these measurements reflect the low temperature phase transition as a change of the observed parameter. From $T_{1\rho}$ results on DPPC,[16] one may confidently assume that the Larmor frequency of 80 MHz lies in the high frequency tail of the relaxation spectrum below the main transition of ethanolamines. Consequently, the higher values of T_1, observed at low temperatures of DMPE, can again be considered as the consequence of the reduced molecular motion in this state.

Before we turned to further investigations by X-ray analysis, it was evident from the ^{1}H-NMR data alone, that the low temperature phase of DMPE and DPPE is different from the "subgel" phase, reported by Wilkinson and Nagle.[14] The experimental evidence is given by the observation that heating from low temperatures to a gel phase temperature (say from -30°C to 20°C) results in the normal M_2-value of this temperature. This behaviour would not occur if the starting point were the subgel phase. This phase is considered stable, and, consequently, it should not be able to transform back into the $L\beta$-phase, once it is formed at low temperatures.

However, we have observed a slow temporal transformation from gel to subgel in DMPE, in accordance to the results of other authors.[13,14] Apparently the kinetics of this transition are temperature-dependent. In terms of the ^{1}H-NMR measurements, the final subgel state is characterized by a second moment close to the rigid lattice value, and of T_1 values of $\sim$1.2 s. So, a second distinction between the subgel and the low temperature phase is indicated by the difference in the spin lattice relaxation times. These results imply that the subgel phase is also restricted in molecular motion, but exhibits a different dynamic spectrum.

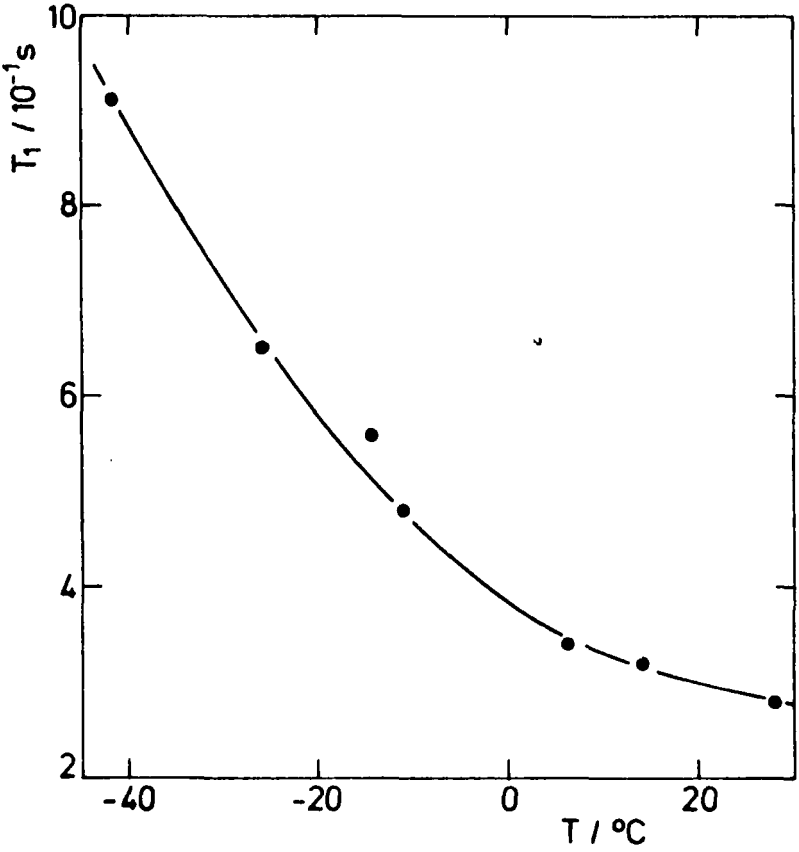

FIGURE 3 Development of the spin-lattice-relaxation time T_1 *vs.* temperature, recorded in DMPE at 80 MHz.

In order to obtain further structural information on the temperature dependent behaviour of ethanolamines, small- and wideangle X-ray diffraction was performed with DMPE at various temperatures ranging from −15 to 55°C. The resulting membrane periods P and the hexagonal d_{100}-spacings of the hydrocarbon region are given in Figure 4. The main transition can clearly be seen around 50°C and is equivalent to the features known from lecithin,[17,23] as there is a broadening of the wide angle reflection due to chain melting and a reduction of the long period by 11%, due to less extended chains. We observe a superposition of Lα and Lβ indicated by double reflections of P, even below the main transition temperature, in accordance to our NMR findings. Below this transition no steps occur as the temperature falls

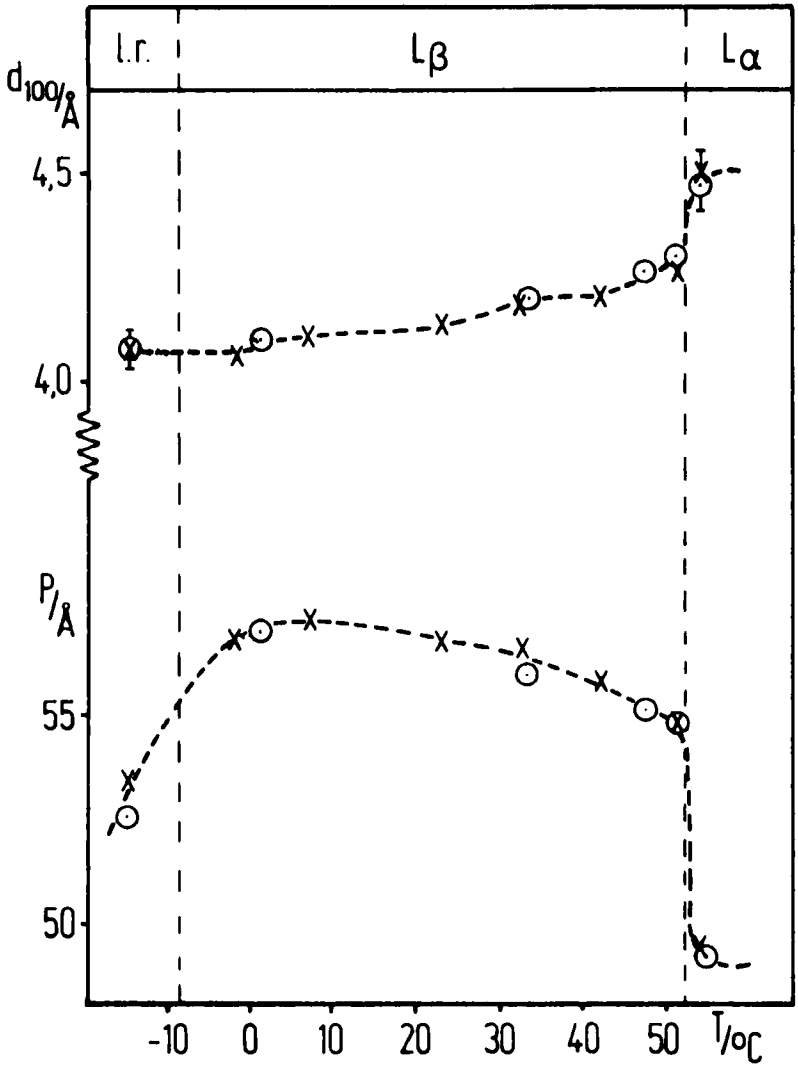

FIGURE 4 Hydrocarbon lattice spacings, d_{100} of hexagonal symmetry and membrane period P *vs.* temperature.
l.r.: low temperature range
x: oriented samples
o: unoriented samples
Error bars indicate width of broadened reflections

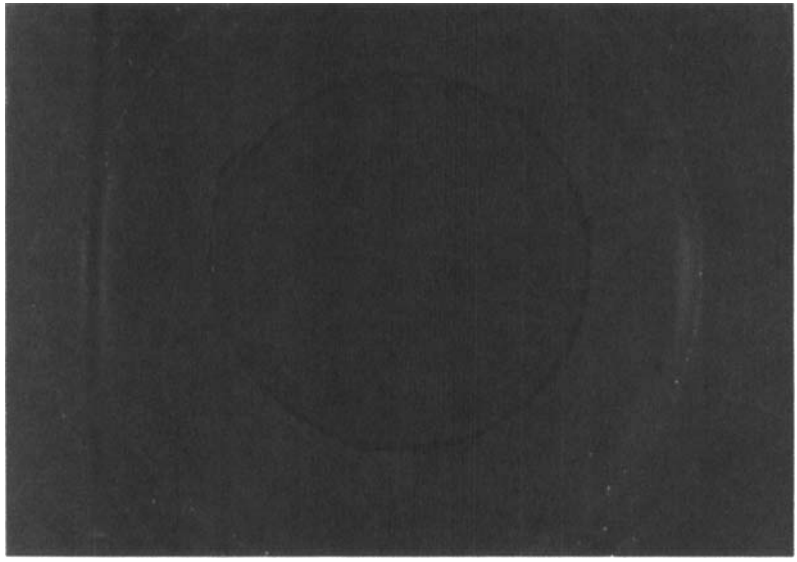

FIGURE 5 Wide angle diffraction of oriented sample of DMPE. Sharp equatorial arcs are similar for all normal Lβ-temperatures. Background scattering due to mylar. Center cut out for small angle scattering at larger film distance.

to −2°C with regard to both spacings, but a continuous decrease in d_{100} and an increase in P. This again quite clearly indicates the absence of a pretransition. The only drastic change occurs in P, which at −15°C is reduced by 10%, whereas d_{100} stays constant, but is broadened by a factor of 2.5. The broadening of the hexagonal reflection of the CH_2-chains is due to reduced long range order, which implies distortions of the first or second kind (after Vainshtein),[24] paracrystals (after Hosemann),[25] or a particle size as small as 50 Å. In the range of −5 to 49°C the appropriate phase assignment should be Lβ as no chain tilt occurs according to our oriented sample diffraction (see Figure 5). The crosses in Figure 4 indicate the oriented, the circles the unoriented, preparation of samples. Both are in good agreement, and correspond to the continuous changes of the ^{1}H-NMR second moments.

The development of the subgel state is characterized by the coexistence of the Lβ-phase reflections and an increasing contribution of a split CH_2-reflection d (Figure 6). This process is accompanied by an intensity transfer from the Lβ-membrane period P to a reduced spacing. At room temperature this transformation takes some 400 h. Interpreting the 3.84 Å reflection as d_{200}, and the 4.08 Å reflection

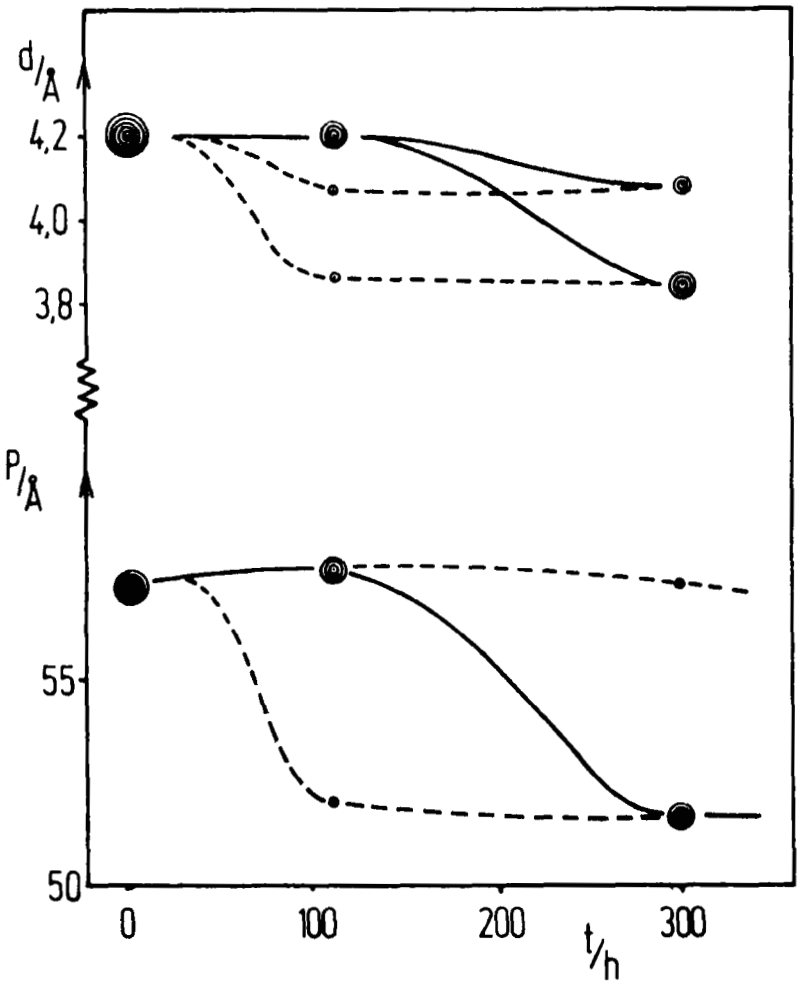

FIGURE 6 Transition Lβ to "subgel" state, demonstrated by intensity transfer of d_{100} and P. Number of circles indicates intensity, as taken from unoriented sample diffraction. Similarities to a paraffin CH_2-lattice appear after 12 days, referring to Lσ′ phase in DPPC.

as d_{110} of an orthorhombic lattice, the values are close to those found in the Lσ′ phase of DPPC,[4-7] and also to the paraffin lattice values.[26] Drying an oriented sample at 50°C gives a similar pattern. However, in this case at least three regions of different states are demonstrated by the coexistence of three membrane repeat distances. It is interesting to note the correlation of the hydrocarbon lattices of several

membranes are evident from the meridional periodicity of the CH_2-reflections.

CONCLUSION

In this work the complex phase behaviour of DMPE- and DPPE-bilayers is demonstrated. One can distinguish four different phases by both, ^{1}H-NMR and X-ray diffraction. In addition to the well known gel and liquid crystalline phases, we observe a transition to a state of substantially reduced molecular mobility at lower temperatures. The chain packing symmetry in this phase is not changed, but its long range order and the interlamellar distance is reduced by some 10%. This state is different from the subgel phase, discovered recently,[12-14] even though some experimental parameters are similar, such as the low hydrocarbon chain mobility and the reduction in the lamellar distance. In accordance to the interpretation of Wilkinson and Nagle,[14] the formation of the subgel phase can be visualized as a continuous nucleation process, where the growth of domains of the densely packed subgel-bilayers takes several hundred hours. From these findings it becomes evident that both new phases are in some ways similar to the Lσ'-phase of DPPC. In the low temperature phase, the freezing of the headgroup rotation, the second moment values, and the magnitude of T_1 are similar to the NMR-findings in the Lσ'-phase of DPPC. In the light of the X-ray measurements (in particular, the splitting of the CH_2-reflections), it is the subgel phase, which appears to be the more closely related to the Lσ'-phase. It will be the subject of further investigation, to explore the relationship between these phases.

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References

1. S. C. Chen, J. M. Sturtevant and B. J. Gaffney, *Proc. Natl. Acad. Sci. USA*, **77**, 5060–5063 (1980).
2. D. G. Cameron and H. H. Mantsch, *Biophys. J.*, **38**, 175–184 (1982).
3. H. L. Casal, H. H. Mantsch, D. G. Cameron and B. P. Gaber, *Chem. Phys. Lip.*, **33**, 109–112 (1983).

4. H. H. Füldner, *Dissertation Ulm*, (1980).
5. H. H. Füldner, *Biochemistry*, **20**, 5707–5710 (1981).
6. M. J. Ruocco and G. G. Shipley, *Biochim. Biophys. Acta*, **684**, 59–66 (1982).
7. M. J. Ruocco and G. G. Shipley, *Biochim. Biophys. Acta*, **691**, 309–320 (1982).
8. P. W. Westerman, M. J. Vaz, L. M. Strenk and J. W. Doane, *Proc. Natl. Acad. Sci. USA*, **79**, 2890–2894 (1982).
9. L. Trahms, W. D. Klabe and E. Boroske, *Biophys. J.*, **42**, 285–293 (1983).
10. J. F. Nagle and D. A. Wilkinson, *Biochemistry*, **21**, 3817–3812 (1982).
11. L. Trahms and W. D. Klabe, *Proc. of the 10th Intern. Liq. Cryst. Conf., Mol. Cryst. Liq. Cryst.*, (1984).
12. H. Chang and R.M. Epand, *Biochim. Biophys. Acta*, **782**, 319–324 (1983).
13. H. H. Mantsch, S. C. Hsi, K. W. Butler and D. G. Cameron, *Biochim. Biophys. Acta*, **782**, 325–330 (1983).
14. D. A. Wilkinson and J. F. Nagle, *Biochemistry*, **23**, 1538–1541 (1984).
15. M. Hentschel, R. Hosemann and W. Helfrich, *Z. Naturforsch*, A. **35**, 643–644 (1980).
16. E. Boroske and L. Trahms, *Biophys. J.*, **42**, 275–283 (1983).
17. M. Hentschel and R. Hosemann, *Mol. Cryst. Liq. Cryst.*, **94**, 291–316 (1983).
18. A. Abragam, *The principles of nuclear magnetism*, Oxford, Clarendon Press (1961).
19. L. Trahms and E. Boroske, *Biochim. Biophys. Acta*, **552**, 189–193 (1979).
20. A. L. MacKay, *Biophys. J.*, **35**, 301–313 (1981).
21. D. Marsh, A. Watts and I.C.P. Smith, *Biochemistry*, **22**, 3023–3026 (1983).
22. J. Seelig, *Quat. Rev. Biophys.*, **10**, 353–418 (1977).
23. A. Tardieu, V. Luzzati and F. C. Reman, *J. Mol. Biol.*, **75**, 711–733 (1973).
24. B. K. Vainshtein, *Diffraction of x-rays by chain molecules. Elsevier Publ. Comp. Amsterdam-London-New York*, (1966).
25. R. Hosemann and S. N. Bagchi. Direct analysis of diffraction by matter, North Holland Publ. Comp., Amsterdam. (1962).
26. C. W. Bunn, *Trans. Farad. Soc.*, **35**, 482 (1939).