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

Lyotropic stress in lipid-water model systems. Structural changes induced by freezing of the solvent

G. Förster[a]; G. Brezesinski[a]
[a] Martin-Luther-Universität, Halle/S., G.D.R.

To cite this Article Förster, G. and Brezesinski, G.(1989) 'Lyotropic stress in lipid-water model systems. Structural changes induced by freezing of the solvent', Liquid Crystals, 5: 6, 1659 — 1668
To link to this Article: DOI: 10.1080/02678298908045676
URL: http://dx.doi.org/10.1080/02678298908045676

LIQUID CRYSTALS, 1989, VOL. 5, NO. 6, 1659–1668

Lyotropic stress in lipid–water model systems
Structural changes induced by freezing of the solvent

by G. FÖRSTER and G. BREZESINSKI
Martin-Luther-Universität Halle-Wittenberg, Sektion Chemie,
DDR-4010 Halle/S., G.D.R.

In a differential scanning calorimetry and X-ray study 12 different lipid–water model systems have been investigated in the temperature region from 250 to 290 K. Structural changes of the lipid system are observed in most cases in the course of freezing or melting of excess water, although the structures of the phases existing around 273 K were quite different. According to thermodynamic considerations a concentration jump (lyotropic stress) is predicted in all cases during the freezing process of excess water. The given lipid–water system reacts to the lyotropic stress in a specific way according to its individual phase diagram.

1. Introduction

Phospholipids are amphiphiles which are insoluble but swell in water. After adding, for example, 50 wt % of water they spontaneously form multilayer liposomes dispersed in excess water. The use of such model membranes has contributed a great deal to the understanding of the behaviour of lipids in biological membranes.

The phase behaviour of phospholipid–water systems is quite complex, as various hydrated phases occur depending on the chemical constitution, temperature and water content. We therefore limited our investigations to the excess water region and examined the influence of the chemical structure of headgroups or hydrophobic chains on the thermal and structural behaviour of the lipid systems [1–3]. As a part of this work we give a general view of the structural behaviour in phospholipid bilayers in the course of freezing or melting of the excess water. Spectroscopic studies indicate that changes in all parts of the lipid molecules take place in this process [4], but only little is known about structural changes in the systems [5, 6].

2. Experimental

2.1. *Materials*

Figure 1 contains the structural formulae, nomenclature and abbreviations of all of the lipids investigated. The substances L1 and L2 were purchased from Fluka (Switzerland) and were used without further purification. The local anaesthetic heptacaine (H) was a gift of Dr Balgavý [7]. The synthesis of the branched chain lipids (D1–D6) has already been described [1, 2]. The resultant crude products were purified by column chromatography.

2.2. *Methods*

The differential scanning calorimetric (D.S.C.) measurements were performed using a Perkin–Elmer (Norwalk, Connecticut, U.S.A.) DSC-2 instrument which was standardized and operated as described in [1]. X-ray diffraction measurements were

0267–8292/89 $3.00

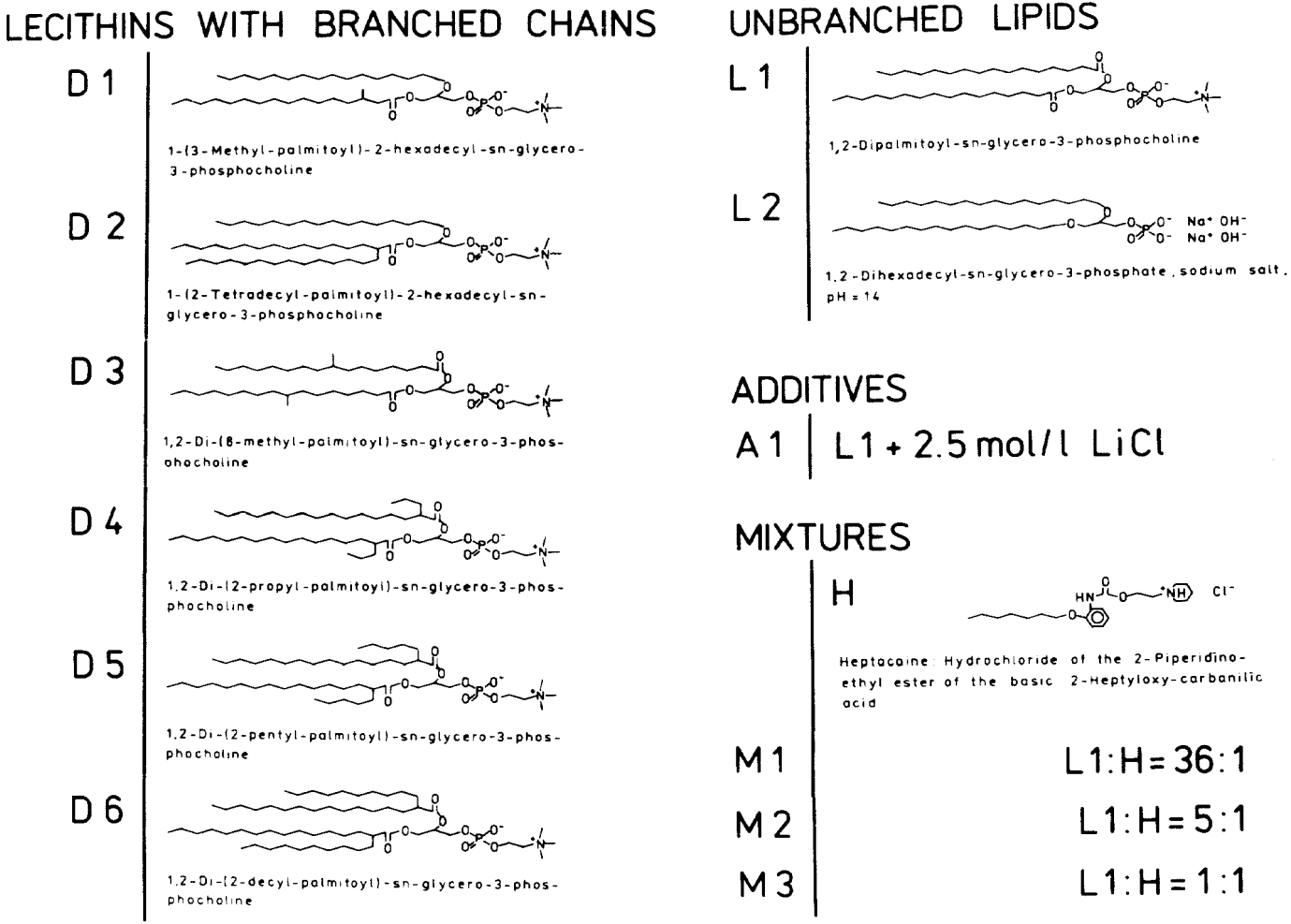

Figure 1. Chemical constitutions, nomenclature and abbreviations of the investigated compounds.

carried out with a powder diffractometer HZG 4 (VEB Präzisionsmechanik Freiberg, G.D.R.) using a transmission technique.

A weighed quantity of the lipid was mixed with excess water (50 wt %). To ensure bilayer formation and hydration all samples were incubated above the main transition temperature of the respective lipid for 1 h. The dispersions were sealed in aluminium D.S.C. pans or in glass capillaries.

Freezing and melting of excess water find expressions in an ice peak in the D.S.C. curves apart from the transition peaks of the lipid phases. The amount of non-freezable (i.e. bound) water, n_B, can be calculated from the quantitative ice peak evaluation [8].

The physical state of the excess water can be derived directly from the X-ray scattering curves. Figure 2 shows as an example two scattering curves (at 266 K) of

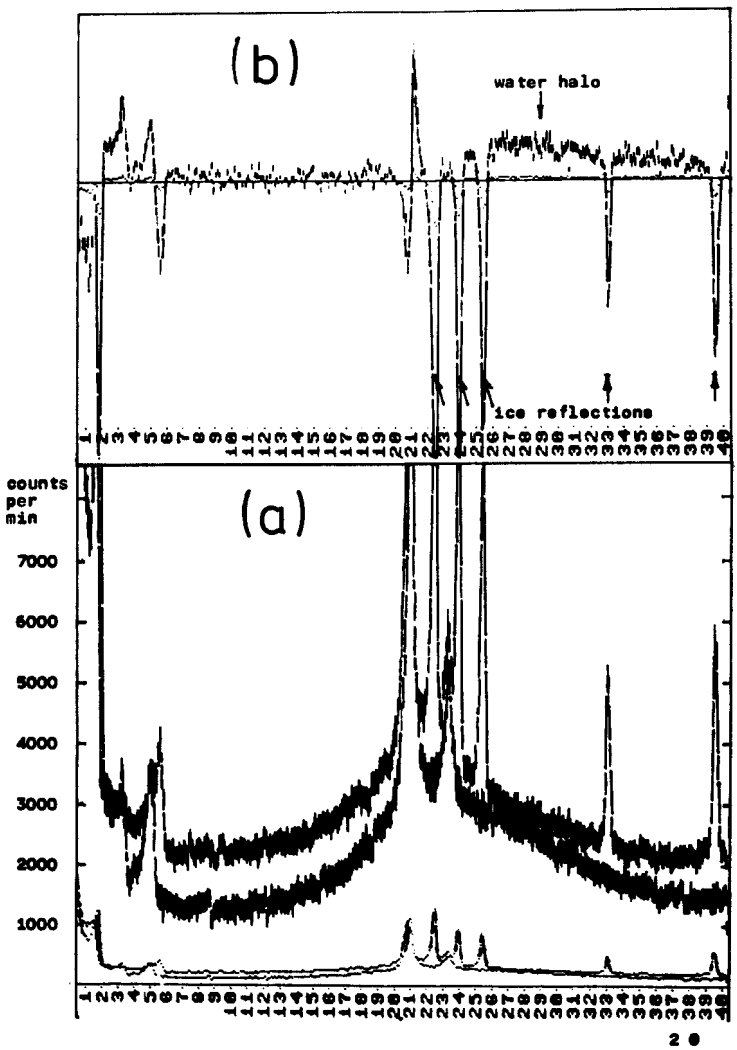

Figure 2. (*a*) X-ray scattering curves of the phase L_{ϱ_i} at 266·4 K with supercooled water (below) and frozen water (above, shifted vertically by 1000 counts): system D1. (*b*) Difference curve of both scattering curves, showing the specific scattering of water (halo), ice (sharp reflections), and changes of long and short spacings of the lipid bilayers.

a sample with supercooled and frozen excess water, and their difference curve. Ice produces sharp reflections from the hexagonal crystal modification [9], and a broad halo centred around $2\theta = 29°$ is typical for excess water. Changes of the lipid phase structure can be deduced from the scattering curves, too: long spacing changes reveal variations of the bilayer separation and short spacing changes give indications of closer chain packing or of the formation of a molecular lattice. In a special case where the bilayer separation changes without varying the chain packing, the amount of removable (i.e. trapped) water, n_T, can be calculated according to

$$n_T = F\Delta\bar{d}_L ZAN_L \times 10^{-21}/M_w,$$

where $\Delta\bar{d}_L$ is the difference in lamellar distances (nm), Z is the number of chains per lipid, A is the area per chain (nm^2), N_L is the Avogadro number and M_w is the molar mass of water ($g\,mol^{-1}$). The factor F is different in bilayers with opposite arranged chains ($F = 1$) or interdigitated chains ($F = 2$).

We distinguish between (*a*) reversible changes in the diffraction patterns and (*b*) changes at which a hysteresis was observed between cooling and heating the sample. In the second case the alteration of the lipid phase structure is induced by the freezing or melting of excess water. The large scale supercooling of pure water is a well known phenomenon [10].

3. Results

The amounts of bound and trapped water obtained by D.S.C and X-ray measurements are summarized in the table. The temperature dependence of the superstructure parameter in the range from 250 to 290 K is presented in figures 3–6. For every example the phase structure or its change during the phase transition is sketched schematically. More details on the structure and phase behaviour of the systems mentioned will be published elsewhere.

Changes in the phospholipid–water systems in the course of freezing or melting of excess water.

Lipid system	Freezing of excess water?	Phase structures involved in the hysteresis process	Induced structural changes	Bound water (mol W : mol L)	Trapped water (mol W : mol L)
L1	Yes	$L\beta^{*\prime}$ (metastable)	Parameter	13	4
A1	Yes	$L\beta'$	None	(*a*)	–
L2	No	$L\beta'$	None	(*b*)	–
M1	Yes	$L\varrho$	Parameter	10	3
M2	Yes	$L\varrho$	Parameter	12	19
M3	Yes	$Lf_i \longleftrightarrow Lf_i$	Parameter	23	29
		$\nwarrow L\beta_i^*$	Phase	(*c*)	(*c*)
D1	Yes	$L\varrho_i$	Parameter	15	6
D2	Yes	$Lk\varrho$	Parameter	11	5
D3	Yes	$L\alpha$	Parameter	15	14
D4	Yes	$L\beta' \longleftrightarrow L\beta_i$	Phase	(*c*)	(*c*)
D5	Yes	Le_i	Parameter	15	14
D6	Yes	$H\alpha$	None	5	–

(*a*) Not determined, because of the influence of LiCl.
(*b*) No freezing of excess water in the temperature region investigated (240–290 K).
(*c*) Not determined, because of the superposition of ice melting and lipid phase transition.

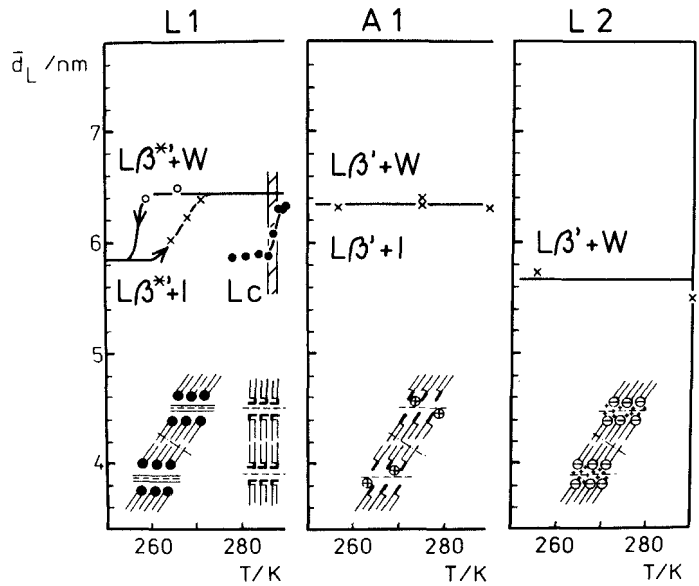

Figure 3. Systems L1, A1 and L2. L1 = 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, A1 = L1 + 2·5 mol l^{-1} LiCl and L2 = 1,2-dihexadecyl-sn-glycero-3-phosphate, sodium salt, pH 14. Temperature dependence of the averaged lamellar distance $\bar{d}_L$ in the range 250–290 K. (x) Values on heating, (○) values on supercooling, (hatched area) reversible phase transition region. Values for L1 are taken from [11] (●) and [5]. Schematic sketches of the phase structure existing at 273 K or its change during the phase transitions are inserted. Nomenclature of the phase notation: W, water; I, ice; superstructure: L, lamellar; H, inverse hexagonal; molecule lattice: type c, e, f; crystallized headgroups: k; chain packing: ϱ, two dimensional orthorhombic; β, two dimensional hexagonal; α, fluid; specifications: i, interdigitated; *, metastable; ′, tilted.

3.1. *System L1 (see figure 3)*

The data from this system are known from the literature [5, 11]. The gel phase Lβ' with tilted chains shows a transition into the subgel phase Lc at 288 K. As the phase Lc develops only under special conditions, usually a metastable phase L$\beta^{*\prime}$ is investigated on cooling the sample [5]. Although the lamellar distance of Lc and L$\beta^{*\prime}$ is the same, no recrystallization takes place.

3.2. *System A1 (see figure 3)*

The addition of 2·5 mol l^{-1} LiCl to L1 does not alter the phase structure. On cooling, excess water freezes but no change of bilayer separation could be detected.

3.3. *System L2 (see figure 3)*

The lipid was dissolved in 1 mol l^{-1} NaOH and exists at pH 14 as the disodium salt. The system shows a gel phase with tilted chains, too, but the content of ions is so high that a freezing point depression prevents the freezing of any water.

3.4. *System M1 (see figure 4)*

The mixture of L1 with the charged local anaesthetic heptacaine shows perpendicular arranged chains in the gel phase Lϱ. They are packed in a two dimensional orthorhombic lattice.

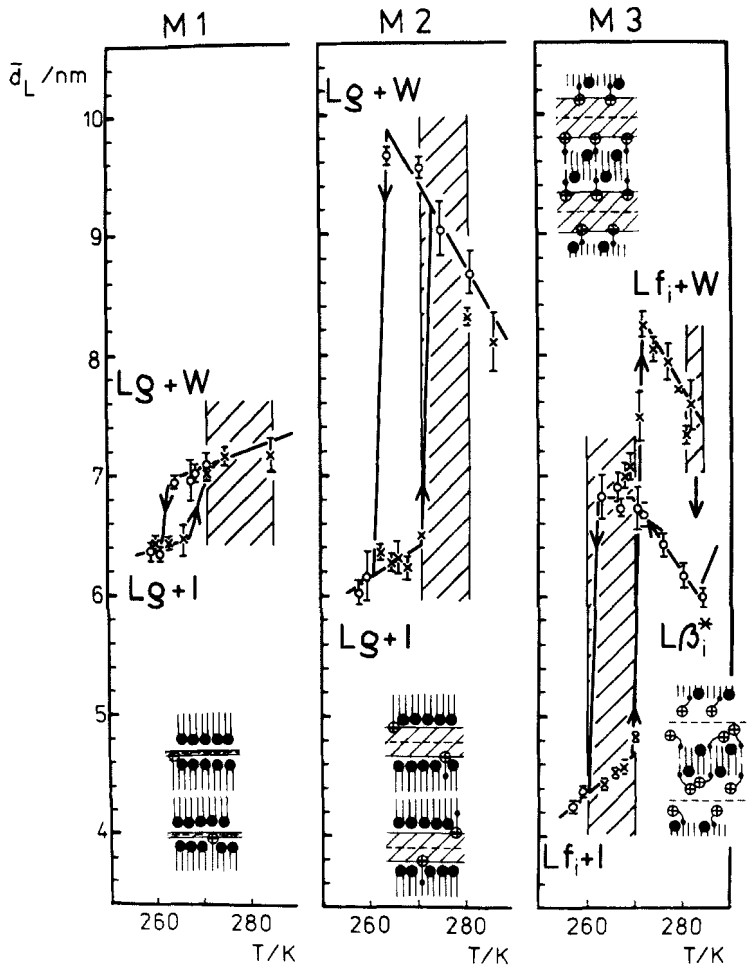

Figure 4. Systems M1, M2 and M3. M1 = L1 : H = 36 : 1; M2 = L1 : H = 5 : 1; M3 = L1 : H = 1 : 1. Temperature dependence of the averaged lamellar distance $\bar{d}_L$ in the range 250–290 K. For further explanations, see the legend to figure 3.

3.5. *System M2 (see figure 4)*

The increase in the concentration of heptacaine also increases the amount of bound and trapped water as a consequence of the higher content of free charges between the headgroups.

3.6. *System M3 (see figure 4)*

The equimolar mixture of L1 and heptacaine shows two specific transition phenomena connected with the freezing and melting of excess water. (*a*) The stable subgel phase Lf_i has a lamellar structure with a molecule lattice in which the chains are orthorhombically packed and interdigitated. It shows a parameter change in the hysteresis process. (*b*) Freezing of excess water out of the metastable phase $L\beta_i^*$ is the only way to attain the stable subgel phase. It induces the phase transition $L\beta_i^* \rightarrow Lf_i$.

It is worth noting that no freezing point depression was observed, which indicates that there is a strong binding of the charges in the headgroup region.

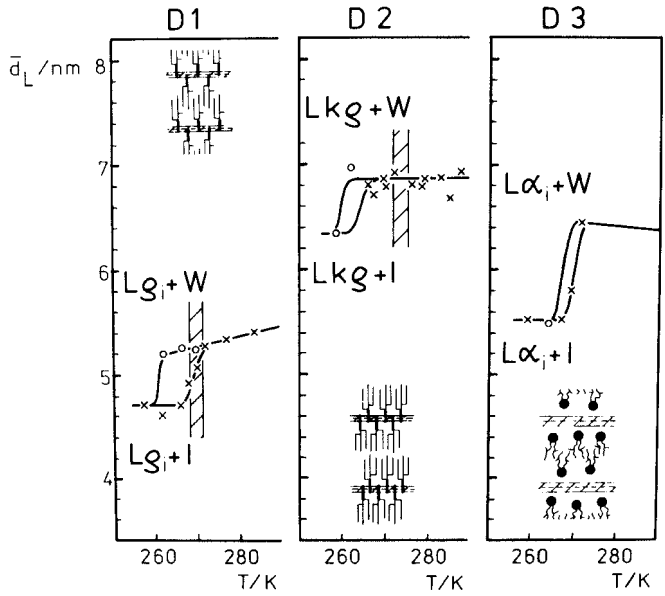

Figure 5. Systems D1, D2 and D3. D1 = 1-(3-methyl-palmitoyl)-2-hexadecyl-sn-glycero-3-phosphocholine. D2 = 1-(2-tetradecyl-palmitoyl)-2-hexadecyl-sn-glycero-3-phosphocholine and D3 = 1,2-di-(8-methyl-palmitoyl)-sn-glycero-3-phosphocholine. Temperature dependence of the averaged lamellar distance $\bar{d}_L$ in the range 250–290 K. For further explanations, see the legend to figure 3.

3.7. *System D1 (see figure 5)*

This derivative is a monobranched lecithin with a short side chain. A gel phase Lϱ_i exists with orthorhombically packed interdigitated chains. This example shows that the introduction of only one short-chain branching into one fatty acid of L1 changes the structure drastically and produces a bilayer structure with interdigitated chains.

3.8. *System D2 (see figure 5)*

In this derivative one long-chain branching is introduced into one fatty acid of L1. A gel phase Lkϱ with orthorhombically packed, opposite arranged chains was found, in which the headgroups are interdigitated and form a two dimensional rectangular lattice. In the hysteresis process trapped water is removable in this structure. At high temperatures the headgroup lattice is sufficiently stable to be preserved even if the chains melt.

3.9. *System D3 (see figure 5)*

This derivative is an example of the fact that short-chain branchings in the middle of the fatty acids suppress the formation of gel phases. With the assumption of opposite arranged chains, too small values for trapped water are estimated. Therefore, bilayers with interdigitated chains are proposed for the phase Lα.

3.10. *System D4 (see figure 6)*

The derivative with a short-chain branching in the 2-position of every fatty acid shows a gel phase Lβ_i in which the interdigitated chains are hexagonally packed.

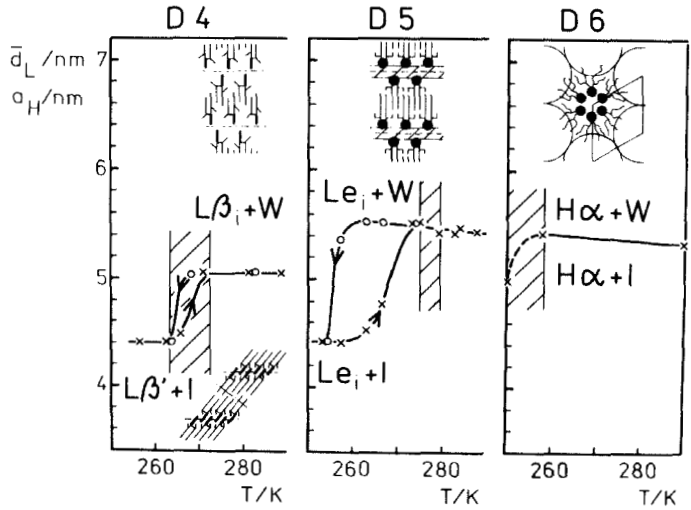

Figure 6. Systems D4, D5 and D6. D4 = 1,2-di-(2-propyl-palmitoyl)-sn-glycero-3-phosphocholine, D5 = 1,2-di-(2-pentyl-palmitoyl)-sn-glycero-3-phosphocholine and D6 = 1,2-di-(2-decyl-palmitoyl)-sn-glycero-3-phosphocholine. Temperature dependence of the superstructure parameters $\bar{d}_L$ (averaged lamellar distance) and a_H (lattice constant of a two dimensional hexagonal lattice) in the range 250–290 K. For further explanations, see the legend to figure 3.

On freezing the excess water, a phase transition to a phase Lβ' with tilted chains occurs [6].

3.11. *System D5 (see figure 6)*

If the chain length of the branching in the 2-position of the fatty acids is elongated, a subgel phase Le$_i$ is observed. It has orthorhombically packed, interdigitated chains and shows a molecule lattice of type e.

3.12. *System D6 (see figure 6)*

The long-chain branchings in the 2-position of the fatty acids decrease remarkably the temperature of the gel–liquid-crystalline phase transition [1]. A liquid-crystalline phase Hα of the inverse type was found. No structural change was detected during the freezing process of excess water.

4. Discussion

The results show that, on freezing excess water, in most cases the phases change their structure parameter without altering the packing within the structural elements. A concentration jump in the bilayers is induced and it can be observed that the phases are thermodynamically stable at lower water content, too. Subgel phases (M3 and D5), gel phases (M1, M2, D1 and D2) and the liquid-crystalline phase Lα (D3) are examples of this group.

In two cases it becomes evident that phases thought to be stable are actually metastable. In the system L1 the lamellar distance of the metastable phase L$\beta^{*\prime}$ after freezing of the excess water is comparable with that of the stable Lc phase. This means that the transition into the thermodynamic stable phase is kinetically controlled. In

contrast, in the system M3 the concentration jump induced by the freezing of excess water leads to the transformation of a metastable phase $L\beta_i^*$ into the stable subgel phase Lf_i.

There were no structural changes in the case of the systems A1 and D6. The high lamellar distance of the system A1 in the presence of LiCl is in agreement with a model derived from nuclear magnetic resonance measurements [12], which suggests an erection of the headgroups. According to this model it may be assumed that, on freezing of excess water, trapped water can be frozen, but steric hindrance of the headgroups prevents a vertical contraction of the bilayers. In the system D6 a liquid-crystalline phase Hα of the inverse type was found. The circular packing of the headgroups in the centre of the cylinders give rise to the speculation that steric hindrance might also be the reason why freezing of excess water does not alter the dimensions of the cylinder. On the other hand, there is no evidence from the methods used that trapped water becomes frozen due to the freezing of free water.

Only in the system D4 does the freezing of the excess water induce a phase transition between two stable lamellar phases. The phase $L\beta_i$ seems to be stable just in the presence of a high water content, whereas the $L\beta'$ phase exists at lower water concentration.

According to thermodynamic considerations the forces between the headgroups determine the equilibrium bilayer separation and the chemical potential of water in the interlamellar space [13]. These depend not only on the chemical structure of the lipid molecule, but also on the phase structure of the system. On freezing the chemical potential of the excess water decreases. Therefore, trapped water is squeezed out of the interlamellar space and crystallizes in holes [14] until a new equilibrium is achieved. Conversely, during the melting process of ice near 273 K water flows back and increases the bilayer separation. The exchange seems to be rather quick. In all cases this thermodynamic model requires removal of trapped water induced by freezing of the excess water. Therefore, with the crystallization of water a sudden concentration jump within bilayers occurs which acts as lyotropic stress and is caused by simple cooling. Every given lipid–water system will react to this stress in a specific way according to its individual phase diagram.

5. Conclusions

The results show that the lyotropic stress forces a lipid–water model system to respond to the concentration jump associated with the freezing or melting of the free solvent. It can easily be realized with all methods and gives more information on the specifity of the system given. However, in not all systems was a structural change detected, although thermodynamic considerations predicted a concentration jump in all of these cases. This demonstrates that D.S.C. and X-ray measurements cannot reveal all phenomena connected with the lyotropic stress. Other methods, e.g. Raman or other spectroscopic methods, should be used in combination.

The authors are grateful to Professor Dr P. Nuhn and Dr B. Dobner for preparing the branched-chain lipids and for valuable discussions.

References

[1] NUHN, P., BREZESINSKI, G., DOBNER, B., FÖRSTER, G., GUTHEIL, M., and DÖRFLER, H.-D., 1986, *Chem. Phys. Lipids*, **39**, 221.

[2] BREZESINSKI, G., DOBNER, B., DÖRFLER, H.-D., FISCHER, M., HAAS, S., and NUHN, P., 1987, *Chem. Phys. Lipids*, **43**, 257.

[3] Dörfler, H.-D., Brezesinski, G., Nuhn, P., Rüger, H. J., and Kertscher, P., 1985, *Colloid polym. Sci.*, **263**, 570.
[4] Casal, H. L., and Mantsch, H. H., 1984, *Biochim. biophys. Acta*, **779**, 381.
[5] Grünert, M., Börngen, L., and Nimtz, G., 1984, *Ber. Bunsenges. phys. Chem.*, **88**, 608.
[6] Förster, G., Brezesinski, G., Bache, I., and Dörfler, H.-D., 1986, *Z. Chem.*, **26**, 28.
[7] Ondrias, K., Horvath, L. I., Balgavý, P., and Stolc, S., 1984, *Physiol. bohemoslov.*, **33**, 489.
[8] Dörfler, H.-D., and Brezesinski, G., 1983, *Colloid polym. Sci.*, **261**, 286.
[9] Dowell, L. G., Moline, S. W., and Rinfret, A. P., 1962, *Biochim. biophys. Acta*, **59**, 158.
[10] Speedy, R. J., 1984, *J. phys. Chem.*, **88**, 3364.
[11] Füldner, H. H., 1981, *Biochemistry*, **20**, 5705.
[12] Lindblom, G., Persson, N., and Arvidson, G., 1976, *Adv. Chem. Ser.*, **152**, 121.
[13] Lis, L. J., McAlister, M., Fuller, N., and Rand, R. P., 1982, *Biophys. J.*, **37**, 657.
[14] Costello, M. J., and Gulik-Krzywicki, T., 1976, *Biochim. biophys. Acta*, **455**, 412.