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X-RAY ANALYSIS AND CALORIMETRY ON PHOSPHATIDYLCHOLINE MODEL MEMBRANES

THE INFLUENCE OF LENGTH AND POSITION OF ACYL CHAINS UPON STRUCTURE AND PHASE BEHAVIOUR

JÜRGEN STÜMPEL, HANSJÖRG EIBL and ALFAR NICKSCH

Max-Planck-Institut für biophysikalische Chemie, Am Fassberg, D-3400 Göttingen (F.R.G.)

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The effect of variation of the acyl chain composition of phosphatidylcholines upon thermal behaviour of multilamellar liposomes was evaluated by calorimetry and X-ray studies. A total of thirteen different phosphatidylcholines were examined. They differed from each other in the length as well as in the position of the acyl chains in the glycerol backbone. The experimental results show that the hitherto accepted phase scheme for phosphatidylcholine-water systems is incomplete and has to be extended to include the behaviour of samples that have been stored for long times at low temperatures. The X-ray results show that the structure of the new low-temperature phase is not in agreement with the hexagonal packing of the acyl chains. To explain the X-ray results, a two-dimensional orthorhombic unit cell has to be assumed in order to fit all the observed reflexes in the wide-angle region.

Introduction

Phospholipids, together with proteins main components of natural membranes, spontaneously form ordered structures in water. For instance the individual shells of multilamellar liposomes have a strong similarity with the lipid bilayer of biological membranes, and it may be hoped that some insights into the many faceted membrane functions may be gained by studying the physical properties of such structures.

Until recently, the thermotropic phase behaviour of multilamellar phosphatidylcholine lipo-

Abbreviations:

1M-2M-PC:	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
1M-2P-PC:	1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine
1M-2S-PC:	1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine
1P-2M-PC:	1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine
1P-2P-PC:	1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine
1P-2S-PC:	1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphocholine
1S-2M-PC:	1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine
1S-2P-PC:	1-stearoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine
1S-2S-PC:	1,2-distearoyl-*sn*-glycero-3-phosphocholine
1M-3M-PC:	1,3-dimyristoyl-glycero-2-phosphocholine
1M-3S-PC:	1-myristoyl-3-stearoyl-*rac*-glycero-2-phosphocholine
1P-3P-PC:	1,3-dipalmitoyl-glycero-2-phosphocholine
1P-3S-PC:	1-palmitoyl-3-stearoyl-*rac*-glycero-2-phosphocholine
T_{s_I}:	temperature of subtransition I
$T_{s_{II}}$:	temperature of subtransition II
T_p:	temperature of pretransition
T_m:	temperature of main transition
ΔH_{s_I}:	free enthalpy of subtransition I
$\Delta H_{s_{II}}$:	free enthalpy of subtransition II
ΔH_p:	free enthalpy of pretransition
ΔH_m:	free enthalpy of main transition

somes in excess water indicated that the phase behaviour of these systems could be classified by the scheme * [1,2]

$$L_\beta(L_{\beta'}) \rightleftharpoons P_\beta(P_{\beta'}) \rightleftharpoons L_\alpha$$

Calorimetrically, two transitions were observed, the so-called main transition attributed to the transition from the $P_\beta(P_{\beta'})$-phase to the L_α-phase, and the so-called pretransition, attributed to the transition from $L_\beta(L_{\beta'})$- to the $P_\beta(P_{\beta'})$-phase. Below the pretransition, X-ray diffraction studies always gave two reflections in the wide-angle region: a sharp and intense reflection and a neighbouring diffuse and less intense one which were assigned to the acyl chain lattice. Besides there, reflections in the small angle range were attributed to the lamellar order (phase).

The phase behaviour of multilamellar phosphatidylcholine liposomes, however, is not only influenced by the variation of acyl chains and their positioning in the glycerol backbone, but also depends upon the preparation and the thermal history of the sample. Recently a third transition, called the subtransition, was found at temperatures below the well-known pretransition, calorimetrically demonstrated for 1P-2P-PC and 1S-2S-PC liposomes. It is noteworthy that the subtransition could be discovered only when the samples were stored for a long time at low temperature [3, 4].

A first indication that the symmetry of the low-temperature phase is not in accordance with the $L_\beta(L_{\beta'})$ structure was obtained from X-ray diffraction studies. Hydrated 1P-2P-PC liposomes which were stored for long times at low temperatures showed some reflections in the wide angle range (10 Å $< d <$ 6.0 Å) which had yet been reported [5,6]. Since, apparently, the scheme

$$L_\beta(L_{\beta'}) \rightleftharpoons P_\beta(P_{\beta'}) \rightleftharpoons L_\alpha$$

only partially describes the phase behaviour of phosphatidylcholine liposomes, we examined several phosphatidylcholines with similar and dissimilar chains using X-ray diffraction and calorimetry. It was our aim to investigate the influence of the variation of chain length in the 1- and 2-positions, and also in the 1- and 3-positions, of the glycerol backbone upon the phase behaviour and microscopic structure.

Materials and Methods

The phosphatidylcholines were synthesized according to published procedures [7]. The positional purity of the fatty acids and of phosphocholine in the respective positions of the glycerol molecules were analyzed as described elsewhere [8] and was better than 98%.

Sample preparation. Multilamellar liposomes for calorimetry and X-ray diffraction were prepared by incubation of 15 mg of lipid with 4 ml of redistilled water at the main transition temperature of the respective lipid for 1–2 h. During incubation the samples were vortexed several times for 1 min. Then, the liposomes were centrifuged at about $1000 \times g$ for 10 min at 4°C. The water content of the resulting pellet was 30–60%.

Calorimetry. The calorimetric measurements were performed with a differential scanning calorimeter (Perkin-Elmer DSC 2 with intracooler). Weighed amounts of the liposomal pellet were sealed in stainless steel pans. The reference pan contained an equivalent volume of distilled water. For each sample several scans were performed between 0 and 10 K above T_m with heating rates of 1.25 K/min in the sensitivity range of 1 mcal/s. Lower heating rates led to the same results. The lipid content of each sample was determined gravimetrically and did not indicate any loss of water during the measurements.

X-ray diffraction. The diffraction studies were carried out using a Guinier-camera. The technical details and the resolution are described elsewhere [9]. The samples were sealed in glass capillaries (2 mm diameter, 0.01 mm thickness; Fa. Hilgenberg, 3509 Malsfeld/F.R.G.). The measurements were done with a thermostatically regulated sample holder in the temperature range between 4 and 5 K above T_m (accuracy 0.5 K). Before each diffraction experiment, which lasted about 10 h, the sample was equilibrated for at least 2 h at the new

* Throughout this paper the tilt angle of the acyl chains to the membrane normal was not estimated. Consequently the primed and unprimed phases were not distinguished.

adjusted temperature. Film analysis was done with a Joyce-Loebl 3 CS Microdensitometer.

Results

Calorimetry

All samples were incubated at 4°C for at least 3 days until 2 weeks and were then transferred immediately into the calorimeter which had been pre-cooled at 0°C. The results of the first heating cycles are summarized in Fig. 1 and Table I.

Considering the results presented the phosphatidylcholines can be divided into two groups. First, the 1,2-phosphatidylcholines with identical acyl chains and second, the mixed-chain 1,2-phosphatidylcholines and all 1,3-compounds. Without the case 1M-2M-PC (see Fig. 1) that will be discussed later, the 1,2-phosphatidylcholines with identical acyl chains are characterized by three transitions: The low temperature transition, called subtransition by others, the pre- and the main transition. The transition temperatures and enthalpies are in agreement with published results [5,3] including the general observation that the subtransition is only observed in the first heating cycle after prolonged storage at low temperature.

In contrast, the mixed-chain 1,2-phosphatidylcholines and all 1,3-compounds show only two phase transitions. The one appearing at the lower temperature depends sensitively upon storage at low temperature and is missing in 1M-2S-PC [10–13] or is drastically reduced in all other cases without precooling. The ΔH values of this transition lie between 4.5 kcal/mol (1P-2S-PC) and 8.8

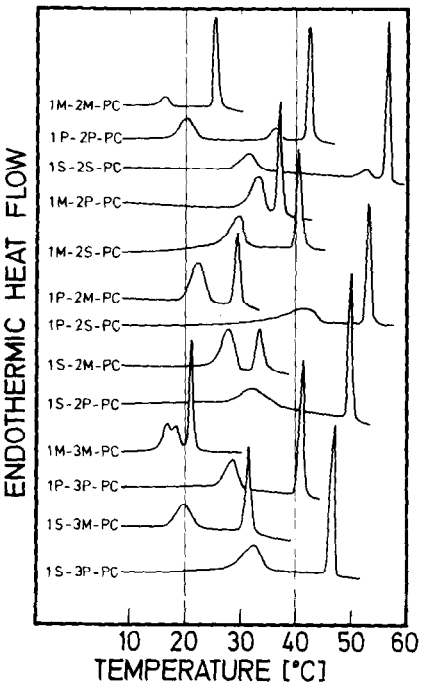

Fig. 1. DSC endotherms of multilamellar phosphatidylcholine liposomes. The first heating curves of annealed samples at 4°C are shown. The heating rate was 1.25 K/min in the sensitivity range of 1 mcal/s.

TABLE I

TRANSITION TEMPERATURES AND ENTHALPIES OF MULTILAMELLAR PHOSPHATIDYLCHOLINE LIPOSOMES

Transition temperatures are presented in °C; enthalpy changes are presented in kcal/mol.

Lipid	T_{s_I}	$T_{s_{II}}$	T_p	T_m	ΔH_{s_I}	$\Delta H_{s_{II}}$	ΔH_p	ΔH_m
1M-2M-PC	–	–	13	23	–	–	1.1	6.3
1P-2P-PC	15	–	34	41	4.1	–	1.6	8.7
1S-2S-PC	27	–	50	56	3.9	–	1.3	10.7
1M-2P-PC	–	30	–	37	–	6.4	–	7.3
1M-2S-PC	–	26	–	42	–	6.6	–	8.2
1P-2M-PC	–	19	–	27	–	8.8	–	6.5
1P-2S-PC	–	35	–	52	–	4.5	–	9.8
1S-2M-PC	–	24	–	33	–	7.9	–	6.0
1S-2P-PC	–	24	–	48	–	5.6	–	8.3
1M-3M-PC	–	15	–	19	–	4.3	–	6.1
1P-3P-PC	–	25	–	39	–	8.0	–	9.4
1M-3S-PC	–	16	–	30	–	5.9	–	7.1
1P-3S-PC	–	26	–	46	–	7.3	–	10.4

kcal/mol (1P-2M-PC) and thus are generally higher than those found for the pretransition in phosphatidylcholines with identical chains (ΔH_p about 1.1 kcal/mol to 1.6 kcal/mol). In one case (1P-2M-PC), the ΔH value of the new transition is even higher than the value for the main transition (8.8 kcal/mol to 6.5 kcal/mol, see also Table I). For comparison, the ΔH values for the main transition with stearic-, palmitic- and myristic acid residues range from 6.1 kcal/mol to 10.4 kcal/mol.

X-ray diffraction

The X-ray diffraction patterns of representative phosphatidylcholines are shown in Fig. 2. At low temperature (5°C) one observes besides several reflections in the 4 Å region two additional ones in the 10 Å $< d <$ 6 Å range, which are for 1P-2P-PC in agreement with the findings of others [5,6].

After heating through the (low temperature) subtransition the two reflections in the 10 Å $< d <$ 6 Å range are no more observable in all cases. In the 4 Å region, however, there are differences due to the acyl chain distribution in the phosphatidylcholines. In the 1,2-phosphatidylcholines with two identical acyl chains (e.g. 1P-2P-PC at 20°C) one observes a sharp and intensive reflection with a less intensive broad shoulder. The patterns of the mixed-chain 1,2-phosphatidylcholines and all 1,3-compounds, on the other hand, show only a single sharp reflection (see Fig. 2: 1M-2S-PC at 26°C, 1P-3P-PC at 36°C).

Having passed the pretransition the patterns of the 1,2-phosphatidylcholines with identical acyl chains coincide with those found in the mixed-chain 1,2-phosphatidylcholines and all 1,3-compounds. Only a single sharp reflection appears in the 4 Å region (see Fig. 2: 1P-2P-PC at 38°C). Further heating through the main transition in all cases leads to a lack of the sharp reflection, instead there appears a very broad one. At low temperature (5°C) the similarity between the different phosphatidylcholines is striking. Only slight deviations exist in the 'long spacings' and in the 'short spacings'. In addition two reflections in the 10 Å $< d <$ 6 Å range are found. It should be mentioned that the lattice constants remain quite

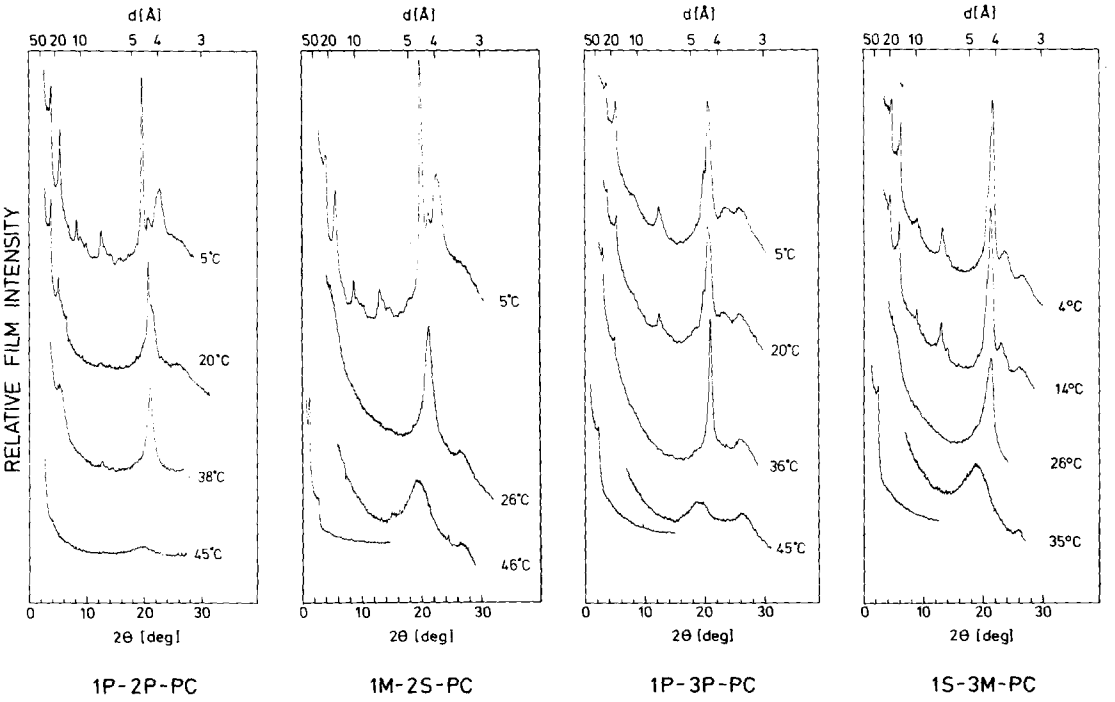

Fig. 2. The X-ray diffraction pattern of representative phosphatidylcholine liposomes. Typical densitometric traces of the observed phases are shown for 1,2- and 1,3-phosphatidylcholines with identical and mixed acyl chains.

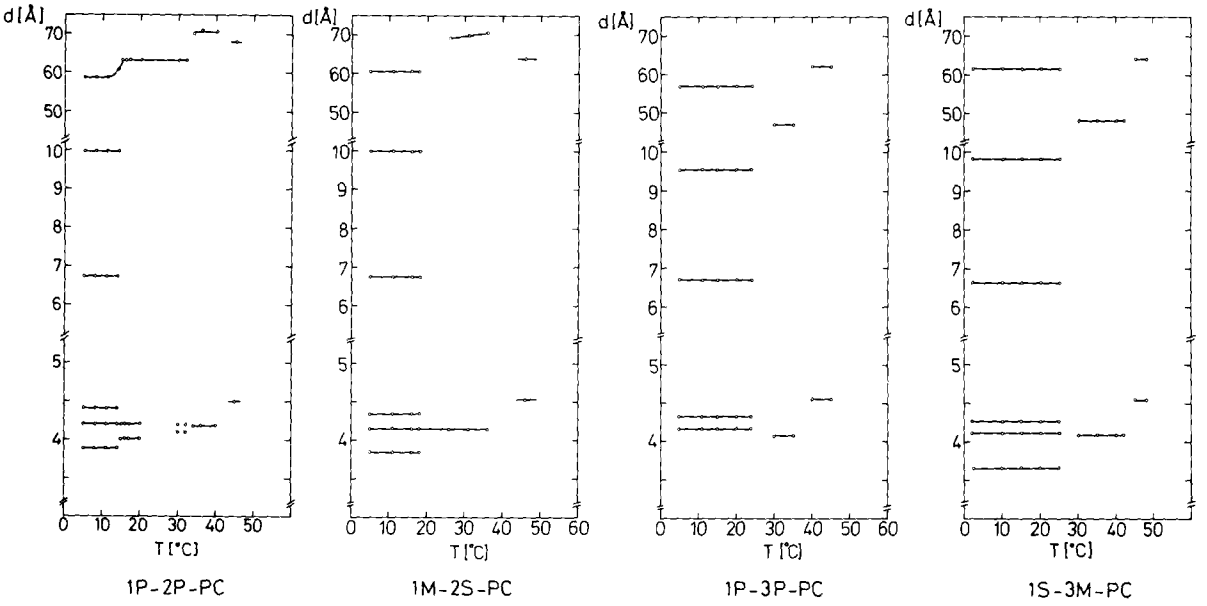

Fig. 3. Temperature behaviour of the spacings in the low and wide angle region. Note that at low temperature (5°C) a pattern is observed which is not found in the $L_{\beta}(L_{\beta'})$-phase.

constant below the low-temperature transition. This similarity is lost in the further thermal 'history'.

At temperatures above the low temperature transition the two reflections in the 10 Å $< d <$ 6 Å range disappeared for all compounds. The 'long spacings' increased in all 1,2-phosphatidylcholines, but decreased remarkably in the 1,3-compounds (see Fig. 3).

For 1P-2P-PC the 4.4 Å spacing is missing; the 4.1 Å reflection stays constant, whereas the 3.88 Å value changes its position to 4.05 Å. This finding, a typical feature of 1,2-phosphatidylcholines with identical acyl chains, was not found in the mixed-chain 1,2-phosphatidylcholines and the 1,3-compounds, where only one reflection remains in the region of 4 Å [14]. This single sharp reflection appears in the 1,2-phosphatidylcholines with identical acyl chains first after having passed the second transition in the gel phase (see Fig. 3: 1P-2P-PC at 40°C). The numerical values of the lattice constants for all phosphatidylcholines investigated are summarized in Table II. Only those values are listed in the table that stay quite constant in the temperature range of the related phase.

Discussion

The calorimetric and X-ray results presented on the thermotropic phase behaviour of annealed multilamellar phosphatidylcholine liposomes in excess water modified in the acyl chain region demonstrate that the known phase scheme only partially describes the thermotropism of phosphatidylcholine model membranes.

The low-temperature phase differs from the symmetry of the $L_{\beta}(L_{\beta'})$-phase with respect to the 4 Å region (three clearly separated reflections were detected in most cases; see Figs. 2 and 3) and with respect to the reflections in the 10 Å $< d <$ 6 Å range, that are not observed in the $L_{\beta}(L_{\beta'})$-phase. It is noteworthy that the distribution of acyl chains in the glycerol backbone of the phosphatidyl molecule makes itself felt only in the kinetics of formation of the $L_{\epsilon}(L_{\epsilon'})$-phase. The occurrence of this phase, however, is independent of the length and of the position of the acyl chains. When the acyl chains in the 1- and 2-position are identical one observes calorimetrically two gel phase transitions (see Fig. 1). The related X-ray diffraction patterns demonstrate that these transitions are accompa-

TABLE II

X-RAY DIFFRACTION DATA OF MULTILAMELLAR PHOSPHATIDYLCHOLINE LIPOSOMES

The temperature dependence of the spacings in the small- and wide-angle region. In the low temperature as well as in the high temperature phase, the higher orders of the 'long spacings' demonstrate that these phases are lamellar. (Data not shown.)

Lipid	T (°C)	d (Å)	s (Å)			
1M-2M-PC	4	58.55	4.17	4.07		
	18	63.85	4.17			
	25	65.40	4.44			
1P-2P-PC	5	58.70	10.00	6.78		
			4.40	4.20	3.88	
	20	63.50	4.20	4.05		
	34	70.00	4.18			
	45	68.00	4.50			
1S-2S-PC	4	64.80	10.10	6.80		
			4.41	4.18	3.88	
	20	66.80	4.42	4.22	3.99	
	40	67.20	4.23	4.13		
	50	70.60	4.18			
	56	69.20	4.55			
1M-2P-PC	5	56.80	7.74	8.07	7.77	
			5.06	4.47	3.95	3.70
	32		4.18			
	36	68.50	4.49			
1M-2S-PC	4	60.40	9.98	6.75		
			4.34	4.15	3.86	
	31	69.90	4.15			
	46	63.80	4.55			
1P-2M-PC	3	55.17	9.96	6.74		
			4.40	3.99	3.84	
	18		4.15			
1P-2S-PC	3	62.70	4.46	3.91	3.67	
	46		4.18			
1S-2M-PC	4	67.30	9.55	4.49	3.72	
	30		4.16			
	39	68.40	4.55			
1S-2P-PC	4	61.40	9.18	8.62	6.70	
			4.47	4.40	3.88	
	23	61.40	9.18	8.62	6.70	
			4.47	3.88		
	40		4.16			
	46	73.60	4.55			
1M-3M-PC	2	52.10	9.73	6.69		
			4.25	4.17	4.07	
	19		4.49			
1P-3P-PC	5	57.04	9.63	6.70	4.14	
	35	47.10	4.12			
	45	62.16	4.55			
1S-3M-PC	5	58.30	9.82	6.69		
			4.33	4.15	3.76	
	20	68.30	4.13			
	35	63.40	4.55			
1S-3P-PC	2	61.60	9.82	6.64		
			4.26	4.11	3.67	
	35	48.20	4.08			
	45	64.20	4.54			

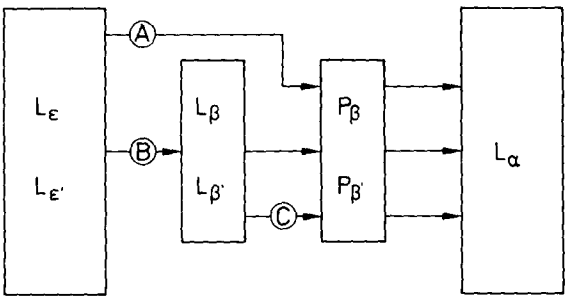

Fig. 4. Extended phase scheme of annealed phosphatidylcholine liposomes between 0°C and T_m. (A) Scheme of mixed-chain 1,2-phosphatidylcholines and all 1,3-compounds. (B) Scheme of 1,2-phosphatidylcholines with identical acyl chains without the exceptional case of 1M-2M-PC. (C) Scheme of 1M-2M-PC.

nied by the symmetry changes $L_\varepsilon(L_{\varepsilon'}) \rightarrow L_\beta(L_{\beta'})$ and $L_\beta(L_{\beta'}) \rightarrow P_\beta(P_{\beta'})$, (e.g. 1P-2P-PC in Figs. 2 and 3).

The other compounds (the mixed-chain 1,2-phosphatidylcholines and all 1,3-phosphatidylcholines), however, show only one gel phase transition (see Fig. 1) which can, according to the X-ray diffraction data, be classified as the symmetry change $L_\varepsilon(L_{\varepsilon'}) \rightarrow P_\beta(P_{\beta'})$ (see Figs. 2 and 3). Thus, the thermotropic behaviour of annealed phosphatidylcholine liposomes in excess water may graphically be expressed as shown in Fig. 4. From this point of view the phase scheme

$L_\varepsilon(L_{\varepsilon'}) \rightarrow L_\beta(L_{\beta'}) \rightleftharpoons P_\beta(P_{\beta'}) \rightleftharpoons L_\alpha$

the feature of the 1,2-phosphatidylcholines with identical acyl chains, represents only an extended case of the simpler scheme

$L_\varepsilon(L_{\varepsilon'}) \rightarrow P_\beta(P_{\beta'}) \rightleftharpoons L_\alpha$

where the additional phase $L_\beta(L_{\beta'})$ is manifested.

Thus, in the temperature range described in this work phosphatidylcholine liposomes apparently are in the $L_\varepsilon(L_{\varepsilon'})$-phase at the low end and in the L_α-phase at the high end of the temperature range investigated. The appearance of the intermediate $L_\beta(L_{\beta'})$- and $P_\beta(P_{\beta'})$-phase is strongly dependent upon the length and the position of the acyl chains in the glycerol backbone *.

* In the mixed chain 1,2-phosphatidylcholines and the 1,3-compounds one cannot exclude that the transition is from $L_\varepsilon(L_{\varepsilon'})$ to L_β. This point will be clarified in a further investigation.

In recent literature [3–5] the appearance of a subtransition has been already described in the case of 1,2-phosphatidylcholines with identical acyl chains. As discussed earlier, the phase below the subtransition corresponds to what we have termed the $L_\varepsilon(L_{\varepsilon'})$-phase. In addition the pretransition and main transition are accepted as transitions from the $L_\beta(L_{\beta'})$- to the $P_\beta(P_{\beta'})$-phase and from the $P_\beta(P_{\beta'})$- to the L_α-phase, respectively. According to Füldner and Ruocco et al. [5,6] subtransition should involve a transition from the $L_\varepsilon(L_{\varepsilon'})$- to the $L_\beta(L_{\beta'})$-phase in the 1,2-phosphatidylcholines with identical acyl chains.

The results described in this paper demonstrate that there are two gel phase transitions possible when starting from the $L_\varepsilon(L_{\varepsilon'})$-phase. For this reason we call the symmetry change from the $L_\varepsilon(L_{\varepsilon'})$- to the $L_\beta(L_{\beta'})$-phase the subtransition I and the change from the $L_\varepsilon(L_{\varepsilon'})$- to the $P_\beta(P_{\beta'})$-phase the subtransition II. Therefore, the subtransition found in the 1,2-phosphatidylcholines with identical acyl chains is the subtransition I whereas the gel phase transition found in the mixed-chain 1,2-phosphatidylcholines and all 1,3-compounds is the subtransition II.

Only with respect to the main transition our results are in agreement with the findings of Chen and Sturtevant [13] and Mason et al. [12]. For the gel phase transition, however, there exist remarkable differences in the transition temperatures and enthalpies which are related to the long storage time at low temperatures. Furthermore it should be remarked that calorimetric analysis of gel phase transitions alone does not allow a definite determination of the lipid phases and may lead to misinterpretation.

In the following we interpret our X-ray data in terms of a two-dimensional membrane lattice. This assumption is reasonable since the 'long spacings' found indicate that there will be no coupling between the lamellar and lateral order. Therefore we have not taken into account the more 'crystalline' phases reported by Tardieu et al. [1] and Ruocco et al. [6] to explain the phase $L_\varepsilon(L_{\varepsilon'})$. For the $L_\beta(L_{\beta'})$-phase the assignment of the membrane lattice has been discussed at length [1,2] under the assumption of independent acyl chains. The motif of the hexagonal or orthorhombic ('distorted hexagonal') unit cell consists of a single acyl chain

TABLE III

CALCULATED AND OBSERVED SPACINGS IN THE WIDE ANGLE REGION FOR THE NEW LOW TEMPERATURE (5°C) PHASE $L_\varepsilon(L_{\varepsilon'})$

The observed spacings fit within a plane orthorhombic unit cell with base vectors $|a| = 9.8$ Å, $|b| = 8.4$ Å and motif co-ordinates (0, 0) and (1/2, 1/4). The systematic extensions, that result in the condition $h + k/2 = 2n + 1$, are shown in parentheses. h, k: Miller indices, n: integer.

	Calculated spacings (Å)						
Indices	(001)	(010)	(011)	(002)	(020)	(012)	(021)
	9.80	(8.40)	6.41	(4.90)	4.20	4.24	3.89
Lipid	Observed spacings (Å)						
1M-2M-PC	–	–	–	–	4.07	–	–
1P-2P-PC	9.93	–	6.74	–	4.23	4.40	4.00
1S-2S-PC	10.10	–	6.80	–	4.18	4.41	3.88
1M-2P-PC	9.74	(8.07)	(7.77)	5.06	–	4.46	3.95
1M-2S-PC	9.98	–	6.75	–	4.15	4.34	3.86
1P-2M-PC	9.96	–	6.74	–	3.99	4.40	3.84
1P-2S-PC	–	–	–	–	3.91	4.46	3.67
1S-2M-PC	9.95	–	–	–	–	4.49	3.72
1S-2P-PC	9.18	(8.62)	6.70	–	4.40	4.47	3.88
1M-3M-PC	9.73	–	6.69	–	4.17	4.25	4.07
1P-3P-PC	9.63	–	6.70	–	–	4.14	–
1S-3M-PC	9.82	–	6.69	–	4.15	4.33	3.76
1S-3P-PC	9.82	–	6.64	–	4.11	4.26	3.76

located at the origin of the lattice. According to the position of the acyl chain with respect to the membrane normal all reflections in the wide angle region can be fit into this cell in consideration of known cristallographic data on fatty acids [15].

The X-ray pattern in the $L_\varepsilon(L_{\varepsilon'})$-phase, however, cannot be explained by independent acyl chains. It is most reasonable, therefore, to assume that the motif consists of at least one whole lipid molecule including the non-independence of the acyl chains. The unit cell proposed in this paper involves an orthorhombic lattice with the base vectors $|a| = 9.8$ Å and $|b| = 8.4$ Å and the motif co-ordinates (0, 0) and (1/2, 1/4) in which the motif is given by two whole lipid molecules. This choice of lattice vectors and motif coordinates takes into account the known chain-chain spacing of 4.7 Å and fits all experimental data. A comparison of the experimentally observed and calculated reflections is shown in Table III. The coincidence of the spacings is convincing. However, for 1M-2M-PC and 1S-2M-PC, either the 10 Å or the 6.8 Å reflection or both were missing. In the case of 1M-2M-PC this is not surprising since no subtransition (expected at temperatures below 0°C) could be calorimetrically observed between 0°C and the main transition temperature. To avoid complications due to freezing of water this temperature range was not investigated. Regarding 1S-2M-PC only the 9.95 Å reflection could be determined for certain. We are planning to do further experiments with more sensitive detection methods to get more information about these exceptional cases.

Concluding it should be remarked that structural studies on model membranes are useful to understand the role of distinct phospholipid molecules in biological membranes. Since the striking observation of Hanahan et al. [16] that natural membranes usually consist of phospholipid molecules with well-defined acyl chain distribution it was hoped to understand the meaning of the preferred positioning of saturated acyl chains in the 1-position and unsaturated ones in the 2-position. In this respect the results reported provide an

understanding about the influence of acyl chain positioning and may lead to a better understanding of molecular parameters responsible for membrane functioning.

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References

1 Tardieu, A., Luzzatti, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733
2 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 254, 6068–6078
3 Chen, S.C., Sturtevant, J.M. and Gaffney, B.J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5060–5063
4 Füldner, H.H. (1980) Ph.D. Thesis, Universität Ulm, Ulm, F.R.G.
5 Füldner, H.H. (1981) Biochemistry 20, 5707–5710
6 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 684, 59–66
7 Eibl, H. (1980) Chem. Phys. Lipids 26, 405–429
8 Eibl, H. and Lands, W.E.M. (1983) Anal. Biochem., in the press
9 Harlos, K. (1978) Biochim. Biophys. Acta 551, 348–355
10 Keough, K.M.W. and Davis, P.J. (1979) Biochemistry 18, 1453–1459
11 Stümpel, J., Nicksch, A. and Eibl, H. (1981) Biochemistry 20, 662–665
12 Mason, T.J., Huang, C.H. and Biltonen, R.L. (1981) Biochemistry 20, 6086–6092
13 Chen, S.C. and Sturtevant, J.M. (1981) Biochemistry 20, 713–718
14 Stümpel, J. (1981) Ph.D. Thesis, TU Brunswick, Brunswick, F.R.G.
15 Malkin, T. (1952) in Progress in the Chemistry of Fats and other Lipids (Holman, R.T., Lindberg, W.O. and Malkin, T., eds.), Vol. 1, p. 1, Pergamon Press
16 Hanahan, D.J., Brockerhoff, H. and Barron, E.J. (1960) J. Biol. Chem. 235, 1917–1923