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Thermodynamic and structural properties of phosphatidylserine bilayer membranes in the presence of lithium ions and protons

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The binding of lithium ions to phosphatidylserine has been studied by differential scanning calorimetry for dialkyl and diacyl lipid forms and by X-ray diffraction for dihexadecylphosphatidylserine (DHPS). On first mixing DHPS with LiCl solutions an ordered L_β (L_c) phase is formed with a bilayer repeat distance of 5.55 nm and one strong wide-angle, chain-chain reflection at 0.405 nm (26°C), corresponding to bilayers of little, (mono)hydrated lipid with chains approximately perpendicular to the membrane surface. On heating, this phase transforms to an inverted hexagonal phase (H_{II}, H_α) with a repeat distance of 3.75 nm, at a chain-melting transition temperature of approximately 90°C (DHPS). Cooling, after equilibration of the $DHPS^- \cdot Li^+$ sample in the fluid phase, creates a new low-temperature phase ($L_{c'}$) which has a repeat distance of 4.0 nm, corresponding to strongly tilted chains ($\varphi = 42°$). The $L_{c'}$ phase also transforms on heating to the H_α phase, but at a considerably lower chain-melting temperature of approx. 70°C (DHPS). The calorimetric behaviour as a function of Li^+ concentration is qualitatively very similar for the different dialkyl- and diacylphosphatidylserines studied, and is analogous to the results obtained on pH titration. After an initial small increase in transition temperature, that is caused by coulombic ion binding and concomitant surface charge neutralization, a much larger increase in the chain-melting transition temperature occurs, caused by dehydration of the lipid, as a consequence of a further stereospecific ion binding. This suggests that Li^+ and H^+ have similar binding sites on the PS headgroup.

Introduction

Despite the recent reports [1,15] on the binding of lithium (Li^+) to lipid bilayers made of phosphatidylserine (PS), the mode, site, and effects of binding of this psychopharmacologically potent ion are still not clear. Here we show that changes caused by the binding of Li^+ to PS^- qualitatively mimic the thermodynamic effect of phosphatidylserine protonation [2]. It seems that Li^+ and H^+ binding affects both the headgroup electrostatics and the headgroup ability to bind water. We describe the changes in the thermodynamic and structural properties of artificial lipid membranes made of various synthetic, disaturated phosphatidylserines after high temperature equilibration and report on the fact that the lipid phase char-

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Abbreviations: DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine; DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine; DDPS, 1,2-didodecyl-*rac*-glycero-3-phospho-L-serine; DTPS, 1,2-ditetradecyl-*rac*-glycero-3-phospho-L-serine; DHPS, 1,2-dihexadecyl-*rac*-glycero-3-phospho-L-serine.

acterized both by crystalline chains oriented perpendicular to the bilayer surface and a very high transition temperature [1], slowly converts upon such equilibration into another phase with lower phase transition temperature and different bilayer structure. Our results indicate that this slow conversion of the $PS^- \cdot H^+$ and $PS^- \cdot Li^+$ complexes is to states which, at least in the case of $PS \cdot Li$, are characterized by a very short lamellar repeat distance. The prerequisite for such change is, however, that H^+ or Li^+ ions were previously allowed to interact with the lipids having fluid hydrocarbon chains. Thermodynamically these states of $PS^- \cdot H^+$ and $PS^- \cdot Li^+$ are nearly identical and correspond in the case of $PS^- \cdot Li^+$ to lamellae of strongly tilted, crystalline hydrocarbon chains. In the unequilibrated state there is similarity with the phosphatidylserine-Ca^{2+} complexes [3,4]. The high temperature, fluid phase of at least $DHPS^- \cdot Li^+$ is of the inverted hexagonal, H_α, type.

Materials and Methods

Phosphatidylserines: 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine (DPPS), 1,2-didodecyl-*rac*-glycero-3-phospho-L-serine (DDPS), 1,2-ditetradecyl-*rac*-glycero-3-phospho-L-serine (DTPS), and 1,2-dihexadecyl-*rac*-glycero-3-phospho-L-serine (DHPS) were synthesized enzymatically according to the procedure of Comfurius and Zwaal [5] from the corresponding phosphatidylcholines or phosphatidylethanolamines. Multibilayer dispersions were prepared from the Na^+- or H^+-salt of PS in 50 mM $(HOCH_2CH_2)_3NHCl$/NaOH (pH 7.5) with 50 μM EDTA and the appropriate amounts of LiCl (Merck, Suprapur) to yield the desired Li^+ concentrations. (For the study of non-preequilibrated samples also the Li^+-salt form of PS was used.) The samples were, unless stated otherwise, preequilibrated at 100–110°C in an oven for $\gtrsim 10$ min. We did not completely standardize this preequilibration time as we have found that the calorimetric results were rather insensitive to it and that the X-ray diffraction data were also strongly affected by other sample parameters (e.g. the degree of crystallinity) that we could not control. We have, however, performed control experiments without high-temperature preequilibration to confirm the results of Hauser and Shipley [1]. In the H^+ binding study, buffers of varying pH and having constant ionic strength ($J = 0.1$) were used, and the proton concentration $[H^+]$ was calculated from the measured pH. Calorimetric experiments were performed with a Perkin-Elmer DSC-2 differential scanning calorimeter equipped with an Intracooler, and X-ray diffraction with a Guinier camera with a bent quartz crystal monochromator selecting the CuK_{α_1}-line ($\lambda = 0.15405$ nm). The same specimens were used for the calorimetric and X-ray diffraction measurements and the lipid purity was assessed by thin-layer chromatography on silicic acid plates using ninhydrin and molybdenum-blue staining procedures, followed by charring. The lipid purity before and after a typical experiment (> 100 min at $T > 80$°C for DHPS) was $> 99\%$ and $> 95\%$, respectively, with phosphatidic acid as the only contaminant in the case of dialkyl-PS and as the main ($> 80\%$) contaminant in the case of diacyl-PS, independent of the solvent system. ($CHCl_3$/CH_3OH/28% NH_4OH (10:10:3, v/v/v) and $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (50:20:7:3, v/v/v/v) were typically used.)

Results

Typical calorimetric scans of dimyristoyl phosphatidylserine dispersions at different pH values were given in Ref. 2, and are not reproduced here. The chain-melting phase transition temperature, T_t, of DMPS dispersions in various buffers starts to increase at quite low ($\sim 10^{-5}$ M) proton concentration, in agreement with the relatively high pK of the PS-carboxylic group. The phase transition temperature measured in the first heating scan with freshly prepared samples is always higher than that determined in the subsequent scans (see Figs. 2 and 4b of Ref. 2) and shifts upwards with increasing proton concentration, finally reaching a value of 84°C for DMPS. This corresponds approximately to the high enthalpy (~ 40 kJ · mol^{-1}), chain-melting phase transition temperature obtained from our DMPS preparations without the addition of water or buffer. If the sample is incubated at $\gtrsim 100$°C before the measurement, transitions at temperatures greater than 52°C are not seen. This latter value for T_t agrees with the

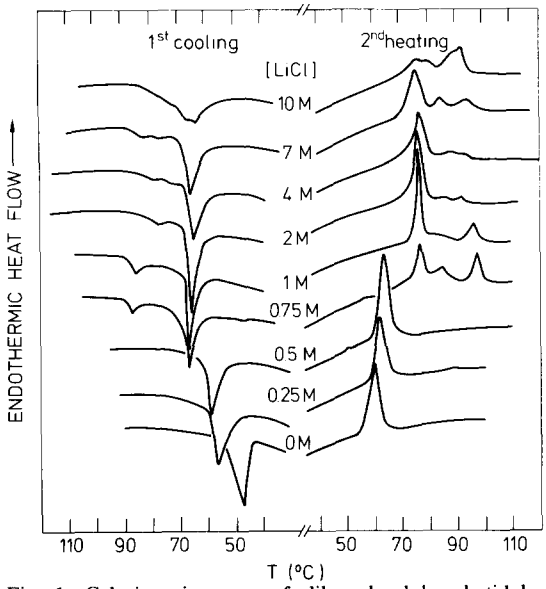

Fig. 1. Calorimetric scans of dihexadecylphosphatidylserine (~ 90 mM, Na salt) dispersed in triethanolamine buffer (I = 0.05, pH 7.5, 50 μM EDTA) containing increasing amounts of LiCl. The first cooling and subsequent heating runs (both at 5 $K \cdot min^{-1}$) are shown for samples kept previously at 100–110°C for > 10 min.

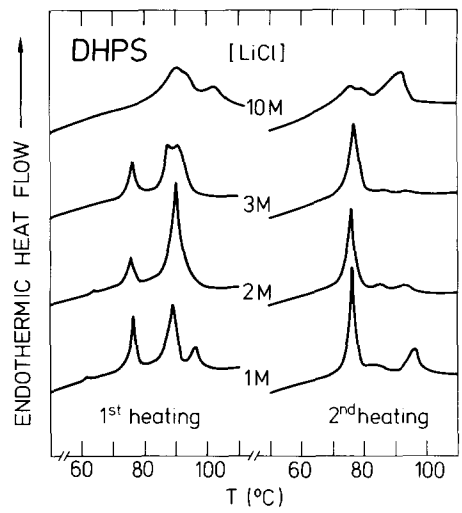

Fig. 2. The influence of the high temperature incubation on the calorimetric recordings of $DHPS^- \cdot Na^+$ dispersions (~ 90 mM) at pH 7.5 and LiCl concentrations of ≥ 1 M. The first heating followed an equilibration at 95°C for 3 min. The second and subsequent heating recordings differ only little and are not markedly affected by the intervening cooling of the sample to 5°C. (All data obtained at 5 $K \cdot min^{-1}$.)

transition temperature reported previously by us (Table I of Ref. 2) and subsequently by Hauser and co-workers [12], if the difference in transition temperature at pH 7.5 of both reports, which may be due to the different primary data analysis methods, is accounted for.

Calorimetric scans of dihexadecylphosphatidylserine dispersions in LiCl solutions of increasing concentrations, which have been preequilibrated at 100–110°C for at least 10 min, are given in Fig. 1. In the low concentration range of LiCl ($\lesssim 0.6$ M) only one main transition is seen up to 100°C with preequilibrated samples (Fig. 1), but additional transitions have been detected at temperatures higher than 90°C for DHPS and higher than 80°C for DMPS, even for low LiCl concentrations, if the samples were not equilibrated at $\gtrsim 100$°C prior to the experiment (see also Ref. 1). For LiCl concentrations greater than 0.6 M, 3–6 transitions are always detected up to quite high temperatures ($\lesssim 84$°C and up to 92°C for DMPS and DHPS, respectively; see Figs. 1 and 2). The second heating scans in Fig. 1 indicate the essential reversibility of the calorimetric behaviour for samples which have been preequilibrated at $\gtrsim 100$°C.

The effects of preequilibration at 100°C or above are illustrated for DHPS in Fig. 2. With samples incubated only at 95°C for 3 min, a large part of the transition enthalpy in the initial scan is associated with the highest melting transition (Fig. 2, 1st heating). In the subsequent scans the peak associated with this transition is reduced in favour of the lowest one (Fig. 2, 2nd heating). This reduction upon heating of the enthalpy associated with the high-temperature transition continues in further scans, but the overall difference between subsequent scans is much smaller. Additionally in experiments with high LiCl concentrations at least two more high-temperature endotherms remain in addition to the main endotherm, even after as many as five temperature cycles (cf. Fig. 1). Furthermore, in very concentrated LiCl solutions (> 6 M), a substantial fraction of the transition enthalpy remains associated with the phase change observed at highest temperatures, and this increases gradually to 100°C upon further increasing the LiCl concentration.

The calorimetrically-determined chain melting

TABLE I

EFFECT OF Li^+ AND H^+ BINDING TO PHOSPHATIDYLSERINE BILAYERS ON THE CHAIN-MELTING PHASE TRANSITION TEMPERATURES [a] (°C)

Lipid	pH = 7.5 [b]	1 M HCl				2 M LiCl		
1,2-Dimyristoyl-PS	36	44	48	(52)	(84)	48	52	(82)
1,2-Dipalmitoyl-PS	54	62	65	(68.5)	(89.5)	64	68	(89)
1,2-Didodecyl-PS	19	36	(40.5)		(71)	36	41	(71)
1,2-Ditetradecyl-PS	41	48	52	(56)	(83)	52	56	(83)
1,2-Dihexadecyl-PS	56	(65)	68	72	(90)	68	73	(92)

[a] Values in brackets denote the transitions that tend to disappear upon successive temperature scans.
[b] Ionic strength was 0.1.

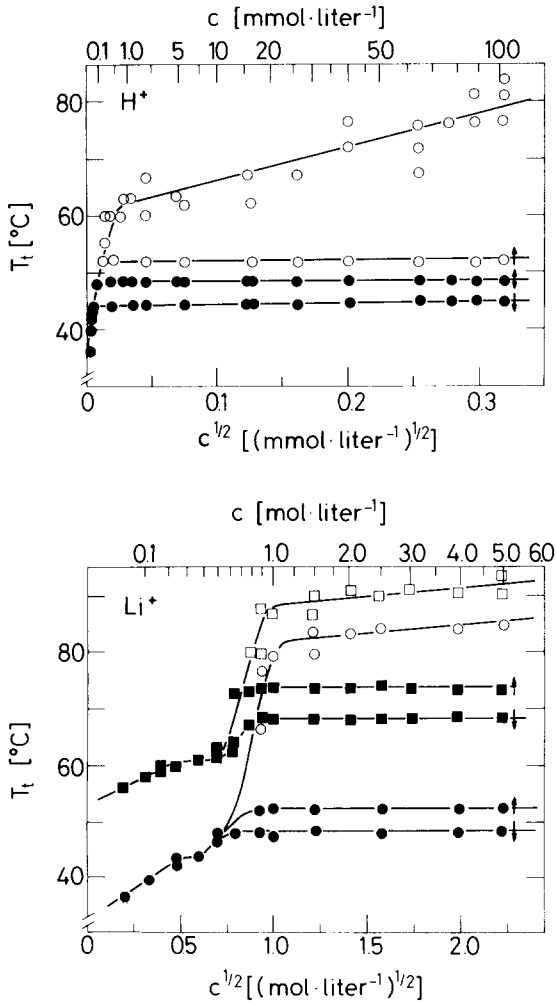

Fig. 3. Effect of the bulk H^+ (upper figure) and Li^+ (lower figure) concentration on the chain-melting phase transition temperature of dimyristoylphosphatidylserine (●, ○) and dihexadecylphosphatidylserine (■, □) membranes. Open symbols denote the transitions that lose intensity (enthalpy) upon repeated heating and cooling of the sample, and filled symbols the transitions that gain intensity and are observed also particularly after preequilibration at 100°C. Transitions seen in one scan only are not shown. Arrows indicate data from the heating (↑) and cooling (↓) scans.

(i.e. high enthalpy) phase transition temperatures, T_t, are given for DMPS as a function of the bulk H^+ concentration and for DMPS and DHPS as a function of the bulk LiCl concentration in Fig. 3, upper and lower graphs, respectively. Both the Li^+- and H^+-induced phase transition shifts are chainlength dependent and, as might be expected, are more pronounced for the shorter chains. These data are summarized in Table I. Also, the qualitative features of the T_t vs. $[Li^+]$ or $[H^+]$ curves do not change with the number of methylene groups per chain or the type of the chain linkage (ester or ether) to the glycerol backbone. This proves that both Li^+ and H^+ induce end-effects and, in comparison with the phase transition shifts observed with other phospholipids [6], one can say that they must be associated with the lipid polar heads.

We were not able to determine the phase transition enthalpies of the various transitions very accurately due to the coexistence of phases. In the case of DHPS the enthalpies of the $T_t \sim 92$°C and $T_t \sim 73$°C phase changes (corresponding for example to the initial and subsequent heatings at [LiCl] > 0.6 M) were estimated to be 53.5 ± 10.5 (12.8 ± 2.5) kJ · mol^{-1} (kcal · mol^{-1}) and 31.5 ± 4 (7.5 ± 1) kJ · mol^{-1} (kcal · mol^{-1}), respectively, and should be compared with the intermediate value measured with the samples at pH 7.5 [LiCl] < 0.2 M of 37.0 ± 2 (8.9 ± 0.5) kJ · mol^{-1} (kcal · mol^{-1}). Also

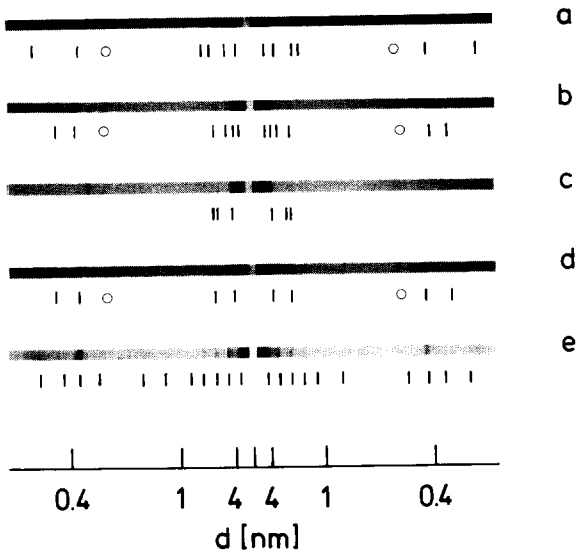

Fig. 4. Typical X-ray diffraction patterns of phosphatidylserine (Na salt) dispersions. (a) 6% DHPS dispersion at pH = 0 and 46°C, after previous heating to 110°C; (b) 6% DHPS dispersion in 3 M LiCl, pH 7.5 at 27°C after 5 min at 100°C; (c) same as (b) but measured at 95°C; (d) 5% DHPS dispersion in 1.5 M LiCl, pH 7.5 buffer at 47°C after high temperature (100–110°C) preequilibration for 30 min; (e) 5% DPPS dispersion in 1 M LiCl, pH 7.5 buffer at 39°C. Lines denoted by ○ are due to the holder.

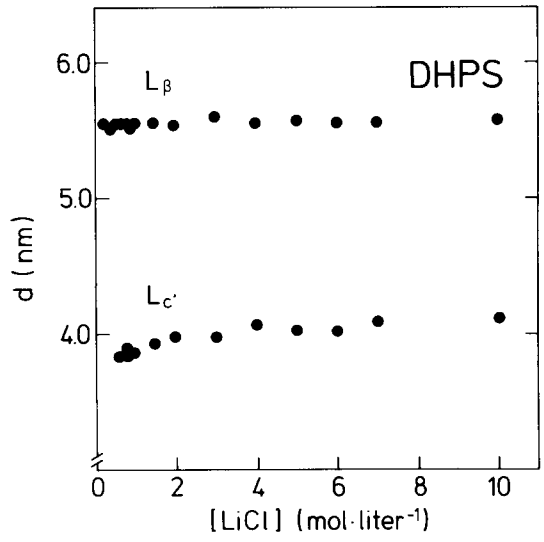

Fig. 5. Lamellar repeat distances of DHPS dispersions (~ 6%) at pH 7.5 as a function of bulk LiCl concentration. The samples were preequilibrated at 100–110°C for > 10 min. The repeat distances of > 6 nm, which were observed for LiCl concentrations < 0.6 M, are not plotted.

with the lipids of other chain lengths, the ratios of the transition enthalpies of the high-temperature and low-temperature transitions for LiCl concentrations greater than 0.6 M were $\gtrsim 1.5$ and tended to be larger for the shorter chains.

In an attempt to clarify the mechanism of ionic binding and its consequences in terms of the structural changes we have investigated the properties of PS · Li complexes also by X-ray diffraction. Because of its greater chemical stability we used for this purpose the dialkyl compound DHPS. Typical X-ray diffraction patterns are given in Fig. 4. We first studied the effect of increasing the Li^+ concentration in excess buffer solution on the lamellar repeat distance, d_r, at 26°C (a temperature well below T_t) with samples that were previously kept for longer than 10 min at 100–110°C. The first reliable diffraction patterns were observed at LiCl concentrations greater than 0.25 M, possibly because our experimental set-up does not permit the determination of repeat distances greater than 11 nm. In the range of LiCl concentrations between 0.25 M and 0.6 M reflections with $d_r = 5.55$ nm were observed, together with others which had repeat distances in the range 6 nm $< d_r <$ 8 nm. The intensity of the former decreased progressively with increasing length of high-temperature preequilibration, but this effect could not be easily quantitated.

At LiCl concentrations greater than 0.6 M, without the prolonged, high-temperature equilibra-

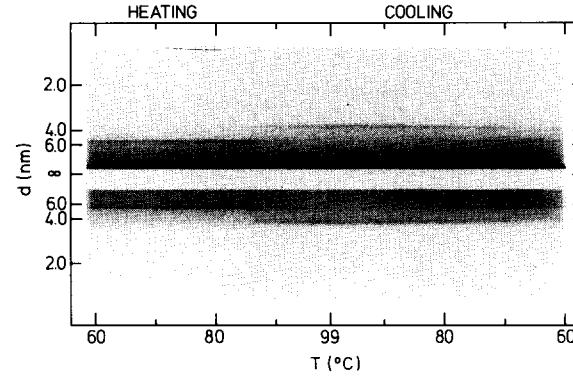

Fig. 6. Continuous variation of the X-ray diffraction pattern of dihexadecylphosphatidylserine dispersions (~ 6%) in 1.5 M LiCl (pH 7.5) as a function of temperature (0.1 K·min^{-1}). Higher order reflections are observed visually on the original film that do not show up clearly in the reproduction.

tion, solely reflections of repeat distance 5.55 nm were seen at low angle (see the continuous temperature scan in Fig. 6, below). This repeat distance was identical within experimental error to the value obtained from nonhydrated DHPS samples, although the number of small-angle reflections was less in the former case than in the latter (four and seven orders, respectively). A single wide-angle reflection at 0.405 nm (at 26°C), that was shifted upon heating towards 0.43 nm (at 85°C), was seen with the majority of our DHPS samples (see Fig. 6, below), indicative of the L_{β}-phase. We do, however, observe several reflections in the wide-angle region of our DPPS samples (at 0.65, 0.47, 0.418, 0.38 (broad), and 0.34 nm, see Fig. 4e) which seem to correspond to the reflections observed by Hauser and co-workers [1] for $NH_4DMPS + LiCl$ (at 0.655, 0.475, 0.418, 0.393, 0.37, and 0.35 nm). With one batch of DHPS (which happened to be *sn*-DHPS) we also detect at least two lines at 0.418 nm and 0.34 nm so that this phase can, in fact, also be of the L_c-type (measurements refer to 47°C, see Fig. 4d).

After high-temperature equilibration a further blurred diffraction line with repeat distance $d_r = 4 \pm 0.1$ nm became visible. This is illustrated in Fig. 5 which gives the LiCl concentration dependence of the repeat spacings for DHPS. The intensity of this $d_r = 4.0$ nm line increased with time of pre-equilibration at above 100°C, at the expense of the $d_r = 5.55$ nm reflection. Unfortunately, only the position of the first order of the $d_r = 4.0$ nm reflection could be determined with accuracy (Fig. 4b, d). Nevertheless, since this is an ordered phase it is safe to assume that it is lamellar. The wide-angle reflection characteristic of this phase also occurred at around 0.42 nm and was very broad. However, upon increasing the LiCl concentration (particularly when $[LiCl] \gtrsim 3$ M) additional lines for example at 0.37 nm appeared, indicative of crystalline chain packing.

In order to assess the influence of possible degradation products, we have prepared samples with phosphatidic acid content ranging from 1 to 10% and determined their thermodynamic and structural parameters without high temperature equilibration. With these specimens we did not observe the phases created by incubating the PS · Li samples at temperatures above 100°C.

Continuous temperature scans of the X-ray diffraction patterns give information on the origin of the various reflections observed at 26°C (see Fig. 6). They have revealed that the phase with the lowest transition temperature corresponds to the structure with the shortest repeat distance ($L_{c'}$), the creation of which is induced by the high-temperature equilibration. The phase with the highest observed chain-melting transition, on the other hand, corresponds to the phase with $d_r = 5.5$ nm (L_{β}). In the case of DHPS the phase with disordered, fluid hydrocarbon chains gave reflections in the ratio 1, $\sqrt{3}$, $\sqrt{7}$, characteristic of the inverted-hexagonal lipid state (H_{α}) [8], with a very short repeat spacing of 3.75 nm (Fig. 4c), implying a low degree of hydration for this phase. Comparison with the results obtained for phosphatidylethanolamines [14] indicates that not more than 2–3 water molecules are associated with each lipid headgroup in the H_{α} phase.

Occasionally, at intermediate Li^+ concentrations ($0.6\ M \leqslant [LiCl] \leqslant 2\ M$) a small part of the sample underwent a transition to the L_{α} phase, as concluded from the appearance of reflections with $d_r = 4.95$ nm and a slightly greater temperature dependence of the repeat distance than for the reflections with $d_r = 5.55$ nm. This transition to the L_{α} phase occurred in the heating run in the temperature range $80°C \leqslant T_t \leqslant 92°C$ (DHPS), but most often at 80°C and/or 86°C. Despite this appearance of the L_{α} phase, at temperatures of 92°C and above, solely the reflections typical of the H_{α} phase were seen. Upon cooling, the H_{α} phase converted to the $L_{c'}$-phase ($T_t \sim 68°C$) or, for a minor part of the sample, to the $L_{\beta}(L_c)$-phase ($72°C \lesssim T_t \lesssim 81°C$). Subsequent heating of the same sample revealed no detectable L_{α}-phase.

Discussion

The non-linearity of the transition temperature shifts as a function of the square root of Li^+ or H^+ concentration (Fig. 3) together with the magnitude of the shifts, indicates that the ion-induced phase behaviour does not arise solely from the screening of the surface electrostatics [2,7]. Because $pK(COO^-) \gg pK(PO_4^-)$ [2], it is reasonable to assume that the initial increase in T_t with increasing LiCl concentration is caused by Li^+

binding to the charged carboxylate group. The Li^+-induced transition temperature shift for DHPS (and less clearly for DMPS) indicates attainment of the full classical electrostatic surface potential screening at concentrations of LiCl $\gtrsim$ 0.6 M [18]. The further increase in transition temperature at Li^+ concentrations greater than 0.6 M demonstrates that, even after the elimination of the net surface charge, Li^+ associates further with the zwitterion of PS^{+-}. It must then interact predominantly with the phosphate group (although it perhaps affects the ammonium groups as well, as suggested by the diminished suceptibility of the PS · Li complex for the ninhydrin staining procedure). The most likely explanation for the non-electrostatic effects is the displacement of (most of) the water of hydration from the headgroup region, caused by the intimate, stereospecific interaction of Li^+ or H^+ with the lipid polar residues (notably the phosphate- and carboxylate-group oxygens). The binding constant for this secondary association is in the region of $K_{Li} \sim 0.8$–1.0 M, assuming the surface electrostatics to be fully neutralized (see Fig. 3). In this respect Li^+ is clearly less effective than H^+ but more effective than Na^+ [2], the binding constant of the latter being $K_{Na} \sim 4$ M.

Our interpretation of the thermotropic phase behaviour of PS · Li complexes is illustrated in Fig. 7, which shows the temperature dependence of the repeat distance of DHPS in LiCl solution of concentrations in the range above 1.5 mol · l^{-1}. The initial state of phosphatidylserine membranes in the presence of excess LiCl solutions is lipid lamellae which are untilted and little hydrated, possibly monohydrated (see later discussion). This form undergoes a transition at 92°C to the inverted-hexagonal phase H_α, also with a very low water content (2–3 H_2O molecules per lipid). On cooling after short equilibration at high temperature (< 10 min) the H_α phase persists until temperatures between 86–68°C, the lower values in the range being observed solely for intermediate salt concentration (0.6 M–1.5 M). The transition is then partly to the $L_\beta(L_c)$-phase with repeat distance $d_r = 5.55$ nm, rather than solely to the $L_{c'}$-phase of repeat distance $d_r = 4.0$ nm; whereas after long incubation at $T > 10$ min) the final state is predominantly of the $L_{c'}$ type and the phase change

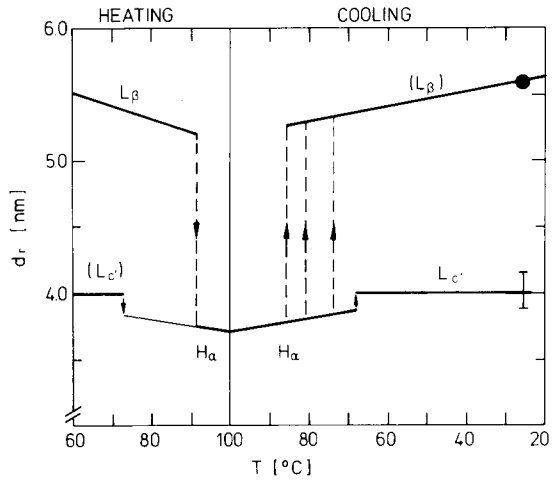

Fig. 7. Schematic representation of the phase behaviour of dihexadecylphosphatidylserine dispersions in the presence of $\gtrsim$ 1.5 M LiCl solution, as reflected in the temperature variation of the X-ray repeat distance. The phases in brackets are seen only with the preequilibrated ($L_{c'}$) or not sufficiently equilibrated samples (L_β). ●: denotes the value corresponding to nonhydrated, crystalline lipid.

occurs at 68°C (cf. Fig. 3). On subsequent heating, the chains of the $L_{c'}$-phase lamellae melt at 72–73°C (DHPS) and hence exhibit some sort of hysteresis. The transition at $\gtrsim$ 90°C is then observed only if at least part of the sample is still in the $L_\beta(L_c)$ phase.

The L_β phase is characterized by a single sharp reflection at 0.405–0.43 nm (26–85°C) in the wide-angle region, suggesting that the chains are untilted in this phase. The identical long spacings ($d_r = 5.55$ nm) of the L_β phase and of the non-hydrated crystalline form suggest that both phases have comparably small tilt angle and that the L_β phase is nearly unhydrated. To check this it is assumed that the chains are all-*trans* and oriented perpendicular to the bilayer surface, giving a hydrocarbon thickness of $d_c = 32 \times 0.127$ nm = 4.06 nm, and an area/molecule equal to twice the chain area of $S_L = 2 \times 0.19$ nm^2 = 0.38 nm^2. (The value of S_L is deduced from the wide-angle chain-chain reflection which occurs at 0.405 nm at 26°C.) The estimated volume of the glycerophosphoserine group is 0.25 nm^3, giving a headgroup thickness of $d_{hg} = 1.36$ nm. If one supposes that just one H_2O molecule is associated with each PS headgroup, this gives an additional contribution of $d_w = 0.16$

nm, and a total bilayer repeat distance of $d_r = d_c + d_{hg} + d_w = 5.58$ nm, in agreement with the measured value. This demonstrates that the crystalline non-hydrated (monohydrate) form is essentially untilted and also that the same conclusion is very likely to be valid for the L_β phase. Here we should note that the formation of an unhydrated ordered bilayer L_β phase for *rac*-DHPS in the presence of Li^+ differs from the behaviour of *sn*-DMPS which was previously observed to adopt an unhydrated crystalline form under these conditions [1]. This difference may be due to the fact that our DHPS was a racemic mixture, as it has recently been found for the phosphatidylcholines that spontaneous crystallization of the chains at low temperatures does not occur with racemic mixtures [17]. Supporting evidence for this statement is that the area per DHPS molecule in the L_β phase is that expected for the crystalline lipid with untilted chains.

The low degree of hydration of the L_β and particularly of the H_α phase implies that the lipid in the $L_{c'}$ phase is also unhydrated or nearly so, as it seems unlikely that the lipid would lose water upon chain melting [14]. The small value of 4.0 nm for the repeat distance in this phase furthermore suggests a large tilt angle for the $L_{c'}$ state. Again assuming monohydration leads to a calculated tilt angle of $\varphi = 42°$ and molecular area of $S_L = 0.54$ nm^2 for the $L_{c'}$ phase ($d_r = 4$ nm), where φ was chosen from the approximately estimated set of sterically permitted tilt angles so as to give the best agreement between measured and calculated repeat distance. Thus both structural and thermodynamic data imply a nearly complete dehydration of the PS · Li complex even in the presence of excess solution. These conclusions are summarized in Fig. 8.

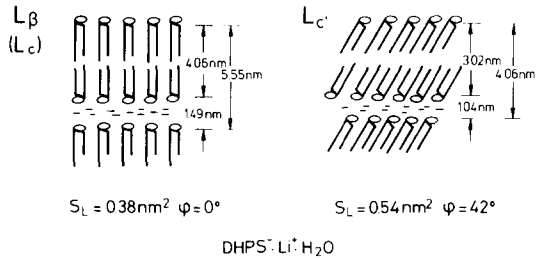

Fig. 8. Schematic indication of the structure of the two possible low-temperature phases of DHPS·Li. The $L_\beta(L_c)$ phase is formed on initially mixing Li^+ with PS^- at low temperature, and the $L_{c'}$ phase is obtained on cooling after incubating at temperatures $\gtrsim 100°C$. Both phases transform to the inverted-hexagonal H_α-phase on heating above the chain-melting transition temperature.

The large chain-tilt in the $L_{c'}$ phase indicates a considerable decrease in the chain-chain interactions between nearest neighbours within each bilayer half, equivalent to approximately three CH_2-groups. If we assume, for the sake of simplicity, that these groups do not contribute to the ordered-phase stability, we might expect that the phase transition of the DHPS · Li complex should occur at a temperature comparable to but slightly higher than that of the anhydrous di-$(CH_2)_{12}$ homologue DDPS [16]. Our measured transition temperature for unhydrated DDPS is ~ 71°C.

To assess, at least approximately, the energetics of Li^+ binding to PS membranes we may use two approaches. Firstly, the binding energy of Li^+ ions to phosphatidylserine bilayers, $G_{Li,b}$, can be estimated from the Li^+ binding constant, K_{Li}. Neglecting the difference between the bulk and interfacial ionic concentration and assuming single-site adsorption (Langmuir type I adsorption isotherm) gives for $K_{Li} \sim 1$ M: $G_{Li,b}(T \sim T_t) \sim -3$ kJ · mol^{-1}, in the case of weak (secondary) stereospecific binding. Secondly, we may use the fact that the binding energy of Li^+ to PS must be larger than or equal to the total binding energy of the displaced water molecules, G_{hyd}, if dehydration is to take place. We may exploit the values that were determined experimentally for G_{hyd} of phosphatidylethanolamine [9,10] and correct them for the effect of the larger PS surface polarity, to get $G_{Li,b}(T > T_t) \gtrsim G_{hyd}(T > T_t) \sim -5$ kJ · mol^{-1} and $G_{Li,b}(T < T_t) \gtrsim G_{hyd}(T < T_t) \sim -3$ kJ · mol^{-1}, above and below the transition, respectively. The circumstance that both methods, despite their approximate character, yield comparable values makes the calculated binding energy at least semi-quantitatively trustworthy.

The qualitative features of the Li^+ binding to PS are independent of the lipid chains. Hence, the increase in $G_{Li,b}$ that occurs at the order-disorder phase transition of the chains is not likely to be due to the existence of the H_α phase per se in the case of DHPS, but rather reflects the better accessibility of some stereospecific binding site for Li^+

on the lipid headgroup in the high-temperature fluid lipid phase. Similarities in the thermodynamic consequences of the Li^+ and H^+ binding suggest that the binding site for these two ions may be identical, involving both the phosphate and carboxylate groups. As a consequence of the possible deep location in the polar region of the latter, this binding site is fully accessible only at $T > T_t$ and leads, once it is occupied, to the increased lateral-area per lipid requirement, for example by inducing a conformational change in the headgroup region. One observation that supports the idea of a Li^+-induced change in the PS-headgroup configuration is the hysteresis of the chain-melting transition, whereby the difference in transition temperature of the heating and cooling runs may be caused by the energy barrier for the change.

It is tempting to believe that our data also provide insight into the basic difference between the thermodynamic properties of the PS complexes with hydrated monovalent ions and those with polyvalent ions. The large chain-melting phase transition shifts observed at low pH or with added Li^+, upon the first heating, qualitatively resemble the changes brought about by binding of Ca^{2+} to PS [3,4,11]. In all of these cases dehydration of the lipid takes place on binding of the highly hydrophilic ion to the lipid polar residues. But whilst the intermolecular bridging capability of Ca^{2+} prevents the loosening of the headgroup region, and chain tilting with concomittant area increase and decrease in transition temperature, in the presence of monovalent alkali ions the PS molecules can become strongly tilted.

The data that we have presented thus strongly suggest that the PS-bilayer state with the high chain-melting phase transition temperature represents only a transiently stable state in the presence of excess, highly concentrated LiCl. When lipid molecules with fluid chains are permitted to interact with Li^+, a complex is slowly formed, that upon cooling yields the phase with the repeat distance $d_r = 4$ nm (DHPS). Whereas the phase we have designated $L_\beta(L_c)$ gives a repeat distance that is to within 0.05 nm identical with that of form I of anhydrous $DPPS^- \cdot H^+$ [12] and equal to that of dry DHPS, the state $L_{c'}$ is a new feature of phosphatidylserine membranes. It is more strongly tilted than the dry, crystalline form II of $DPPS^- \cdot H^+$ [12] (the repeat distances are 4.0 nm and 4.6 nm, respectively) and, concluding from the calorimetric data, it is independent of chainlength and chain linkage. It is a consequence of the intimate, stereospecific but weak binding of Li^+ to the phosphatidylserine headgroup (binding energy in the order of -3 kJ $\cdot$ mol^{-1}) in a site that can also be occupied much more easily by H^+, and which is fully accessible only in the phases with fluid chains. After the report on metastability of dilauroylphosphatidylethanolamine [13] the PS $\cdot$ Li complexes thus represent another example of slow transitions between various possible lipid states that can occur in fluid lipid bilayers and are hence of potential biological relevance.

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