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The metastability of saturated phosphatidylcholines depends on the acyl chain length

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We report the peak temperatures and enthalpies of the main (gel to liquid crystal) transitions and 's-transitions' of symmetric saturated PC's (C_nPC) with n varying from 12 to 22, where s-transition is defined as the transition between metastable and stable phases. The line for s-transition peak temperature versus n ($t = 1.98n - 16.0°C$) crosses the corresponding pre- and main-transition curves between $n = 12$ and 14. We also find that the metastability pattern for lipids of n less than 14 is different to that for n greater than 14 (and is similar to that of the phosphatidylethanolamine analogs). For n greater than 14 the enthalpy of the s-transition decreases with increasing n and extrapolates to zero above $n = 20$, implying that the s-(orthorhombic) and gel (quasi-hexagonal) phases become identical. That simultaneous gel-liquid crystal transition and metastability is restricted to the range $n = 12$ to 20 may be correlated with the predominance in natural lipids of these acyl chain lengths.

Phospholipids play a critical role in the functional and structural properties of biological membranes. However, characterization of this role is made difficult by the fact that biological membranes contain a complex mixture of such lipids. One approach to this problem is to study the properties of simple model systems such as liposomes composed of well-defined phospholipids. A characteristic feature of such multimolecular lipid arrays is their capacity to undergo phase changes, the nature of which is determined by such variables as state of hydration, length and saturation of the acyl chains, and structure of the polar head group. Recently it has been observed (Ref. 1 and references therein) that phases thought to be stable are actually metastable and that under appropriate conditions the lipid reverts to a less hydrated, closely packed arrangement. These metastable states have been best defined in the saturated phosphatidylcholines and ethanolamines. For example, when an aqueous dispersion of $C_{16}PC$ is stored for three or more days at a temperature around 0°C, a new transition can be detected (e.g. by DSC) at about 20°C. We propose the term s-transition for this transition to or from the stable (e.g. subgel) phase seen after incubation, and a metastable phase, rather than the term 'sub'-transition [1]; the term can also be used for similar transitions in the phosphatidylethanolamines [2]. This s-transition is apparent only on the first heating cycle and reformation requires the lipid to be re-stored in the cold for a further three or more days. In this study we report the effect of acyl chain length on the metastability properties (as

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Abbreviations: DSC, differential scanning calorimetry; C_nPC, symmetric saturated 1,2-diacyl-*sn*-glycero-3-phosphocholine of n carbons per acyl chain.

measured by DSC) of saturated phosphatidylcholines.

The phospholipids were obtained from Avanti Inc. (Birmingham, AL), except that C_{18}PC and C_{22}PC were from Sigma Chemical Co. (St. Louis, MO). The purity of the lipids was established [3] by a single spot on thin-layer chromatography and by the narrowness of the main DSC transition, both before and after cold storage. Dispersions of 0.14 M were formed directly in double-distilled water, with vortexing above the main transition temperature and, after encapsulation in aluminum DSC pans, were stored at 2–6°C. Thermal measurements were made on a Mettler TA2000B DSC [4–6] at a scan rate of 1.2 Cdeg/min. Calibration was based on indium, and graphical areas measured by planimetry [4]; masses were determined gravimetrically and by phosphate assay.

Fig. 1 illustrates representative DSC scans of C_{12}PC, C_{13}PC and C_{19}PC dispersions which had been stored in the cold as indicated. C_{19}PC dispersions display the typical three endotherms on the first heating cycle with peak temperatures of 26.9, 57.4 and 60.3°C. Only the latter two endotherms are apparent on a second heating cycle. We have shown [3] similar scans for $n = 16$, 17 and 18. This metastable behaviour is typical of PC dispersions with chains longer than 14, i.e. development of new transitions at temperatures below that of the pretransition. All these lipids show metastability of the gel L_{β} phase below a certain temperature. However, C_{14}PC dispersions stored in the cold initially display only two endotherms [1] with peak temperatures of 17.7 and 24.5°C. The low temperature endotherm disappears on a second heating cycle and is replaced by the 'usual' pre-transition at 15.8°C. This metastability pattern in C_{14}P is seen even in 58 wt% lipid/water dispersions stored in the cold for 455 days. The lipid C_{14}PC thus shows metastability of the L_{β} phase, with the s-transition possibly representing a direct change between the more stable subgel phase and the ripple or P_{β} phase.

Dispersions of C_{13}PC typically display two endotherms on the first heating cycle as illustrated in Fig. 1, at 9.7 and 14.1°C. A second heating cycle shows a reproducible single sharp endotherm at 14.1°C. No pre-transition was demonstrated for this lipid. In one sample (not illustrated) the first heating scan demonstrated a broad endotherm at 14°C which on the second and subsequent scans was replaced by a much sharper endotherm at the same temperature. Thus under certain conditions C_{13}PC can convert directly from the liquid crystal to the subgel phase.

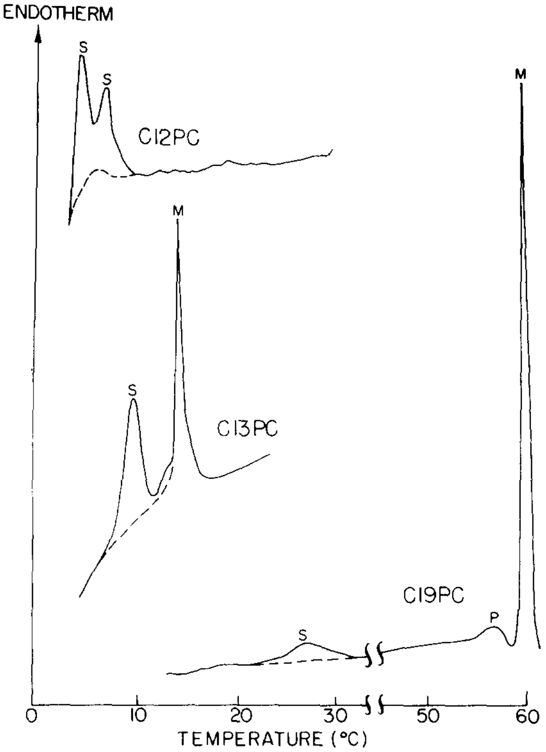

Fig. 1. DSC scans of aqueous liposomes of saturated diacylphosphatidylcholines of number n carbons per acyl chain of 12, 13 and 19, stored at 2–6°C for 70, 9 and 59 days, respectively. The first DSC scan is shown as a solid line tracing, a second scan differs only at the dotted line. As described in the text, C_{12}PC dispersed in 35% v/v ethylene glycol displayed an apparent main transition at −1.1°C.

A complex phase behaviour is illustrated by C_{12}PC dispersions in Fig. 1. The main transition occurs at about −1°C and hence is usually buried inthe ice endotherm. Detection of this endotherm required the addition of cryoprotectant to the aqueous phase. A sample dispersed in ethylene glycol-water shows a sharp endotherm at −1°C (not illustrated) which is consistent with literature values for the main transition [7]. The main transition has been observed in supercooled water at 0°C [8]. It is interesting that a higher temperature shoulder is also apparent in aqueous liposomes which is reproducible on subsequent scans and has previously been observed in aqueous [8] and glycol [9] suspensions. Stored aqueous dispersions of $C_{12}PC$ (with no ethylene glycol) show two endotherms at 3.8 and 6.1°C which are apparent only on the first heating scan. A series of 'nibbling' experiments [3] showed that the 6.1°C transition was independent of the 3.8°C one. Hence $C_{12}PC$ displays liquid crystal (L_α) phase metastability, a previously unreported property for a saturated phosphatidylcholine. The propensity of the L_α phase of $C_{12}PC$ dispersions to be metastable is analogous to the metastable behaviour of the L_α phase of short chain saturated phosphatidylethanolamine (PE) dispersions such as $C_{12}PE$ [2].

Fig. 2 summarizes our results for the peak temperatures of the main, pre- and s-transitions for phosphatidylcholines of acyl chain length 12 to 22, and are perhaps the most comprehensive made in any one laboratory. The temperatures are a continuous function of acyl chain length n. It is also apparent that the dependence of s-transition temperature on acyl chain length is quite low ($t = 1.98n - 16.0$°C) as compared to those for the main and pre-transition. These results considerably expand on our range of observations [1] for the phase properties of a series of mixtures between $C_{14}PC$ and $C_{16}PC$. It is of interest that the complex behaviour discussed earlier with Fig. 1, for n less than about 14, was predicted from the shape of the lines (Fig. 2) above $n = 14$, since they extrapolated to the now confirmed crossing of the s-transition line with the main- and pre-curves.

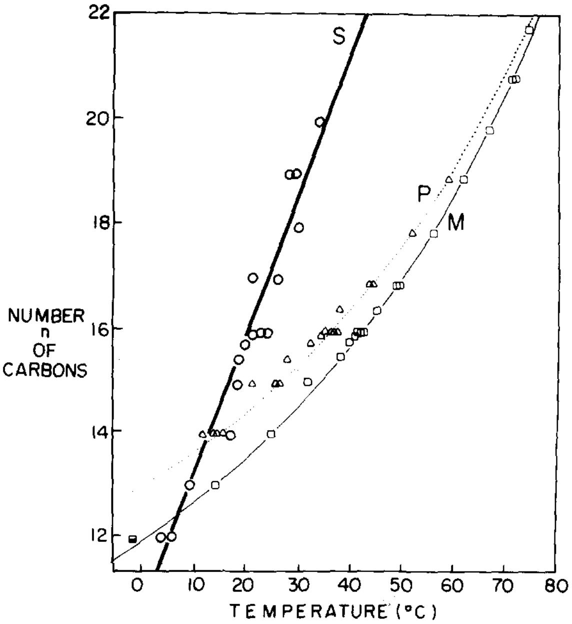

Fig. 2. For aqueous liposomes of saturated diacylphosphatidylcholines C_nPC of number n carbons per acyl chain, the temperatures of the peaks of the main (M), s-(S) and pre-transition(P) are shown. The apparent M-transition for $C_{12}PC$ is that seen in ethylene glycol-water dispersions. The values between 15 and 16 are for mixtures of $C_{14}PC$ and $C_{16}PC$ previously reported [1].

Fig. 3 is a plot of the enthalpies ΔH of the main and new s-transitions as a function of acyl chain length n. The slope of the line relating ΔH (main) to n is positive and has a value of 1.16 kcal/mol per two CH_2 groups — quite consistent with published results [10]. However, for the s-transition for $n = 14$–20, ΔH(s) versus n gives a negative slope. It is curious that the sum of the enthalpies ΔH(main) + ΔH(s) is almost constant between $n = 14$ and 20. Extrapolation of the s-transition curve implies that C_nPC with $n > 20$ will not show metastability. (The s-transition for $C_{20}PC$ is only 0.08 kcal/mol, and was seen after 152 days of cold storage.) Consistent with this prediction we have not observed an s-transition in dispersions of $C_{21}PC$ or $C_{22}PC$ even after 273 and 74 days, respectively, storage in the cold. The main transition line extrapolates to $\Delta H = 0$ at about $n = 9$,

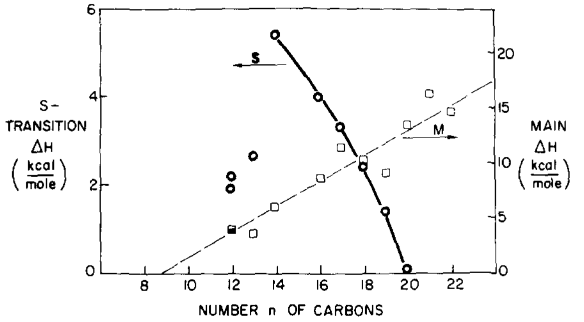

Fig. 3. For aqueous liposomes of saturated diacylphosphatidylcholines C_nPC of n carbons per acyl chain, the enthalpy of the s- (S) and main (M) transitions are given. The line for the M-curve ($\Delta H = 1.16\ n - 10.1$ kcal/mol) is that of a compilation [10]. The enhalpy for $C_{17}PC$ is the sum of the two s-transitions [3]. The apparent main transition for $C_{12}PC$ is that seen in ethylene glycol-water dispersions.

implying that at this value there is then no acyl chain co-operativity. Stable vesicles do not exist for $n < 9$ [11], but we find that $C_{10}PC$ forms stable dispersions with vesicles readily visualized by freeze-fracture microscopy. Hence a conjecture [9] — that a ΔH versus transition temperature plot implies no bilayers for n less than 12 — does not hold.

We sought metastability in many samples of $C_{11}PC$ dispersions in water, and obtained results of unsatisfactory reproducibility. Dispersions formed in 35% v/v ethylene glycol have been left in the cold for up to eight months. We have observed a small endotherm at $-18°C$ consistent with the main, but no metastable phases. Ethylene glycol at this concentration does not prevent formation of the typical s-transition in $C_{16}PC$ dispersions [1]. Thus it would appear that only C_nPC's with $n = 12$ to 20 display metastability.

Generally, reversion of a lipid in its metastable region to a new stable crystalline-like subgel phase involves two processes: dehydration of the polar head group region and rearrangement of the acyl chains into a close packed mode. The thermodynamic stability of the new phase will depend upon a delicate balance between these two processes. We propose that, as n increases, the acyl chain packing modes in the crystalline and gel phases converge and the energy difference between the modes vanishes. This is reasonable in light of the small crystallographic difference between the quasi-hexagonal gel and the orthorhombic subgel phases [12]. Conversely, as n approaches 9, chain-chain interactions become reduced and a close crystal-like gel packing arrangement is no longer possible for the phospholipid bilayer.

Thus, for a bilayer of saturated phosphatidylcholines to show simultaneously metastability and main transition, the acyl chain length must lie between 12 and 20. It is of interest that biological membranes are composed predominantly of lipids with acyl chains of this length.

Finally, we have begun to define the role of water in the manifestation of metastability. $C_{16}PC$ was 'dispersed' with trehalose rather than water following Crowe et al. [13]. Such a $C_{16}PC$/trehalose mixture displayed a s-, pre- and main transition at 34, 43.4, and 46.1°C respectively. Only the pre- (not reported earlier [13]) and main transitions were apparent on the second and subsequent DSC scans. No ice endotherm was observed. This observation suggests that the critical interaction, at least in the head group region, is the formation of hydrogen bonds between the phosphate head group and OH groups and that water itself is not essential for s-transitions.

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