Biochimica et Biophysica Acta 816 (1985) 303–312
Elsevier

BBA 72635

Permeability and morphology of low temperature phases in bilayers of single and of mixtures of phosphatidylcholines

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(Received January 31st, 1985)

Key words: Phospholipid bilayer; Differential scanning calorimetry; Membrane permeability; Freeze-fracture; Phase transition; Surface morphology

The properties of subtransitions were studied in aqueous dispersions of saturated phosphatidylcholines (PC) by means of permeability measurements, freeze-fracture electron microscopy, and differential scanning calorimetry (DSC). For dispersions of C_{16}PC, a C_{16}PC analog (2,3-dipalmitoyl-cyclopentano-1-phosphocholine with four methylene residues between the nitrogen and the phosphorus atoms) and C_{17}PC, there was good agreement between phase properties (including subtransitions) as observed by DSC and temperature-related permeability. C_{16}PC and C_{17}PC dispersions also displayed a 'crinkled' surface morphology in the subgel state. The phase diagram for mixtures of C_{14}PC and C_{16}PC was consistent with ideal mixing of these two components in the subgel state and also illustrated the relative independence of the subtransition on acyl chain length as compared to the pre- and main transitions. Together, these results indicate that (i) permeability, DSC and freeze-fracture electron microscopy measurements do correlate reasonably well with the existence of a subgel state, (ii) mixtures of lipids with similar acyl chain lengths can be used to investigate subtransitions, (iii) the development of a subtransition appears to be mainly a function of the non-acyl chain moiety of the phospholipid.

Introduction

A standard model for biomembranes is the multilamellar bilayer formed by phospholipids and known as the liposome [1,2]. Liposomes are of recent interest as potential drug carriers [3]. When aqueous dispersions of saturated phosphatidylcholines are stored for three or more days at a temperature around 0°C, new phases are observed at temperatures below the pre-transition. These 'subtransitions' have been detected by many techniques including DSC [4], dilatometry [5] and phosphorus NMR [6], and the packing arrangement of the phase below the subtransition characterized (as orthorhombic) by X-ray diffraction measurements [7]. These low temperature phases are slow to form and are apparent only on the first heating cycle. The re-appearance of the subtransition requires the lipid to be re-stored in the cold for a further three or more days. Parenthetically, similar phases have also been observed for saturated phosphatidylethanolamines [8–10] and phosphatidylcholine analogues [11] in which glycerol has been replaced by cyclopentanetriol. In this study we address three questions with regard to these low temperature phases. (a) How do the barrier properties of aqueous dispersions in the subgel phase compare with those of the gel phase

Abbreviations: C_nPC diacylphosphatidylcholine, of n carbons per saturated acyl chain; FWHM, full width at half maximum; DPCyPC 2,3-dipalmitoylcyclopentano-1-phosphocholine with four methylene residues between the nitrogen and the phosphorus atoms.

and do such dispersions display a local permeability maximum on passing through a subtransition temperature, analogous to the permeability maximum observed on passing through the main (gel to liquid crystal) transition? (b) For mixtures of phosphatidylcholines, such as $C_{14}PC/C_{16}PC$ what does the phase diagram indicate about the miscibility of the two components in the subgel phase? (c) Are there morphogical correlates, as visualized by freeze-fracture microscopy, to the packing arrangement below the subtransition as has been described for one of the cyclopentanetriol analogues of dipalmitoylphosphatidylcholine?

Methods

The phospholipids used in this study (dimyristoyl $C_{14}PC$, dipalmitoyl $C_{16}PC$, diheptadecanoyl $C_{17}PC$, distearoyl $C_{18}PC$) were obtained from Sigma Chemical Company (St. Louis, MO). DPCyPC was a gift from Dr. A.J. Hancock [11]. Lipids were considered pure as judged by a single spot on TLC as well as the narrowness of the main transition endotherms on DSC scans, both at the time of preparation and after several months storage in the cold. We have already discussed questions of purity [12]. Multilamellar liposomes were formed by techniques well established in this laboratory [13] which involve evaporation of lipid from chloroform into a thin layer. Dispersions were formed in double distilled water. DSC was performed using a Mettler TA 2000 B modular thermal analysis system [14,15,16] at a scan rate of 1.2 deg. C/min. 20 μl of lipid dispersion were pipetted into each aluminum pan to give approx. 2 mg lipid. Lipid dispersions in pans were stored in a refrigerator which has a temperature range of 2–6 deg. C.

Vesicles to be used for permeability (efflux) measurements were prepared by drying down an appropriate aliquot of a stock chloroform solution of a given lipid and dispersing the dried lipid in 50 mM NaCl/5 mM Tris-HCl/^{22}NaCl (3 μCi/ml), pH 7.5, above the lipid transition temperature. The vesicles were dialyzed overnight at 5°C against buffer lacking the isotope to remove untrapped tracer, and then incubated at different temperatures in stoppered glass tubes to measure the efflux rates [13].

It is possible that during the overnight dialysis step, trapped as well as untrapped isotope would be removed. Loss of the former would depend upon the permeability of the vesicle to ^{22}Na. To evaluate this possibility we measured the internal capture volume of these liposomes by counting lipid samples after overnight dialysis. From a knowledge of the amount of isotope trapped by the liposomes and the concentration of isotope in the original swelling solution the internal volume (or capture volume) in μl/μmol lipid can be calculated [17]. Values quoted in the literature vary from 0.34 to 4 μl/μmol lipid [18,19] although the 'true' internal volume of phosphatidylcholine (multilamellar) liposomes is unknown. In Bangham's original paper [1] the capture volume for egg phosphatidylcholine was 0.7 μl/μmol lipid. We had previously measured the capture volume for a series of saturated phosphatidylcholines after overnight dialysis using ^{22}Na and [^{14}C]glucose as markers [17]. The values ranged from 0.6 for $C_{14}PC$ to 1.4 (μl/μmol lipid) for $C_{18}PC$ and were equal for both markers. The DPCyPC used in the present experiments had a capture volume (^{22}Na) of 0.7 μl/μmol lipid. These internal volumes are consistent with those reported in the literature and indicate that a significant fraction of the trapped isotope is retained during the overnight dialysis step. Furthermore, the equality of glucose and sodium trap volumes is evidence against any specific (electrostatic) binding of sodium to the head-group region. As a further test of the dialysis step, we have compared for $C_{16}PC$ the ^{22}Na internal volume after overnight dialysis with the ^{22}Na internal volume measured by removing untrapped isotope by repeated centrifugation and washing of the liposome pellet. Both values were in good agreement, being 0.8 μl/μmol lipid (dialysis) and 0.7 μl/μmol lipid (centrifugation).

However, although some trapped isotope will undoubtedly be lost during the overnight dialysis step, the technique used in these experiments does measure actual sodium permeability. Some of the evidence for this includes the differential loss of glucose and sodium from vesicles prepared in this manner from certain lipids [17], the sensitivity of the sodium loss to incorporation of a small amount of an ionophore such as nigericin [17], and finally the large loss of solute occurring specifically at the

main gel to liquid-crystal phase transition (this study and Ref. 17). These observations are most consistent with the ^{22}Na data, reflecting the actual leak rate of sodium across the bilayer rather than some property such as binding.

Freeze-fracture microscopy was done with an Edwards freeze-fracture and etching unit. Vesicles were prepared in distilled water containing glycerol 25% (v/v). The latter is used to reduce crystal formation, since some etching does occur during this procedure. The samples were maintained at temperatures as indicated in the figures until the time of quenching. Grids were examined in a Hitachi model Hs-9 electron microscope.

Although we have attempted to perform all these experiments under identical conditions, such is not truly the case. Buffer (NaCl-Tris) was used for the permeability experiments to eliminate isotopic effects. In the case of DSC and freeze-fracture microsocopy, distilled water was used as the dispersing medium both to simplify the techniques and to eliminate any salt effects during the rapid freezing step of the microscopy work. We did run several DSC scans for $C_{16}PC$ dispersed in buffer (NaCl-Tris) and stored in the cold: these scans were indistinguishable from those run for identical lipid samples dispersed in distilled water. Also, it is unlikely that the buffer would have any significant influence on the structure of these electrically neutral lipids. Bangham et al. [20] showed that low angle X-ray diffraction measurements on egg phosphatidylcholine yielded a constant repeat distance over a wide range of salt concentrations.

Results

Permeability measurements

Fig. 1 illustrates permeability versus temperature profiles for $C_{16}PC$, $C_{17}PC$, $C_{18}PC$ and one of the cyclopentanetriol analogues of $C_{16}PC$ (DPCyPC). All of these dispersion display a permeability increase in the temperature vicinity of the main gel to liquid crystal transition, as defined by DSC (Fig. 2 and Ref. 11; Fig. 3 lowest curve). For the glycerol-based lipids ($C_{16}PC$, $C_{17}PC$, $C_{18}PC$) there is an inverse relationship between the extent of the permeability change at the main gel to liquid crystal transition and the length of the acyl chain. Storage of these aqueous dispersions in the cold for prolonged periods results in alterations in their permeability properties. Firstly, some type of annealing process appears to occur such that the 'baseline' permeability at temperatures below the main transition decreases with storage. This phenomenon is most evident with dispersions of $C_{16}PC$ and its cyclopentanetriol analogue DPCyPC and less apparent with $C_{17}PC$ and $C_{18}PC$. Whether this decrease in permeability is strictly time-dependent or also requires storage at a low temperature is unclear from these experiments. Secondly, stored dispersions display permeability peaks at temperatures below the main transition. For $C_{17}PC$, $C_{16}PC$, and the $C_{16}PC$ analogue DPCyPC, these peak(s) occur at temperatures which correspond closely to those of the subtransitions as measured by DSC. It should be noted that for both $C_{16}PC$ and $C_{17}PC$ the position of the low-temperature permeability peaks for the two separate series do differ by about 4 deg. C. In addition, for $C_{17}PC$ the main permeability rise also onsets at a slightly lower temperature for one of the series compared to the other. The reasons for this observation are not clear but probably reflect both the temperature tolerance of the water baths (± 1 deg. C) plus some variation in liposome preparation. DSC substransitions similarily show slight variations. For $C_{17}PC$ and DPCyPC dispersions these permeability 'bumps' begin to appear after overnight dialysis in the cold. $C_{18}PC$ dispersions display two peaks, at 26°C and 36°C which do not correspond to a DSC-measured transition. However, it should be appreciated that the permeability and DSC measurements do necessarily involve somewhat different experimental protocols. For example, DSC measurements are obtained by scanning the dispersion at a constant rate of 1.2 deg. C/min. In the case of a permeability measurement, after overnight dialysis at 5°C, the dispersion is quickly heated (within minutes) to the desired temperature and then held there for the duration of the experiment (180 min). Since kinetic factors are clearly important in the formation of low temperature phases it is quite possible that permeability and calorimetric measurements detect different aspects of the same underlying phenomenon.

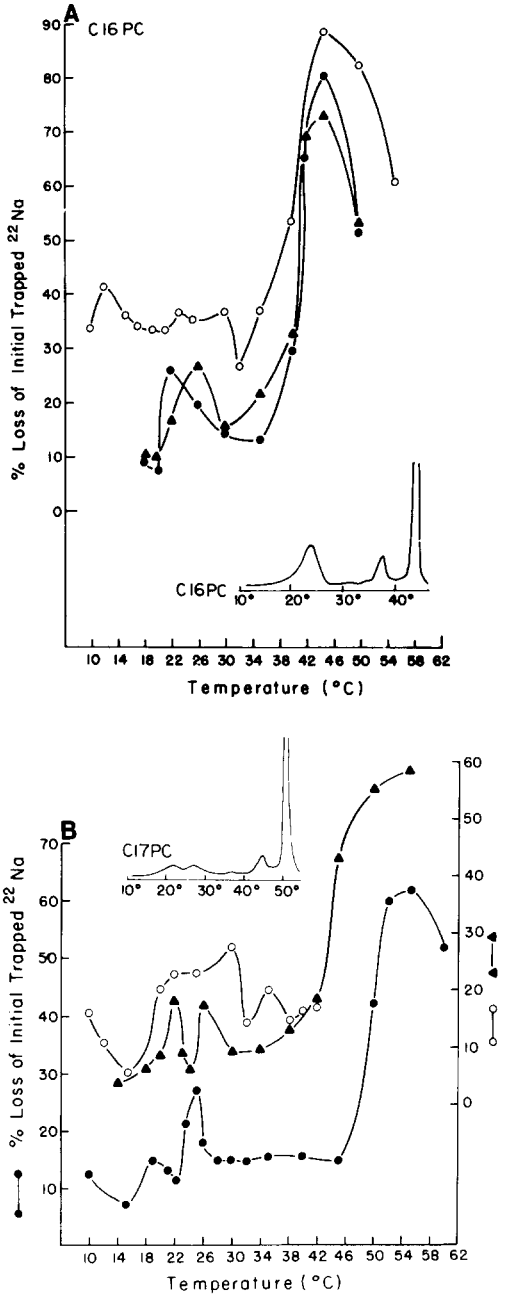
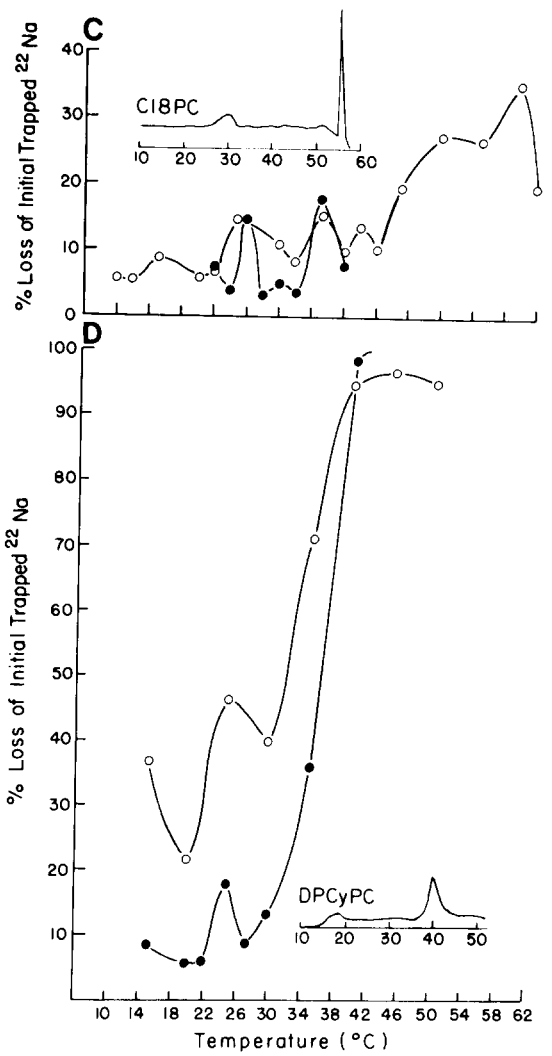


Fig. 1. ^{22}Na effluxes in different vesicles at different temperatures. Vesicles at different temperatures. Vesicles composed of the lipids indicated in the figure were swollen in 50 mM NaCl/5 mM Tris-HCl/^{22}NaCl (pH 7.5). The ordinate refers to the percentage of initial trapped ^{22}Na lost over 180 min (3 h). Experimental points for each temperature represent one, or in some cases two, separate experiment(s), each performed in triplicate. The results illustrated are the mean values. To simplify the figure, error bars have not been given but the range of values did not exceed 25% for any of the mean values. A, $C_{16}PC$. ○, liposomes not stored in the cold prior to overnight dialysis at 5°C. ● and ▲, liposomes stored for 21 days at 2–6°C prior to overnight dialysis at 5°C (results of two separate series of experiments). B, $C_{17}PC$. ○, liposomes not stored in the cold prior to overnight dialysis at 5°C. ● and ▲, liposomes stored for 14 days at 2–6°C prior to overnight dialysis (results of two separate series of experiments). C, $C_{18}PC$. ○, liposomes not stored in the cold prior to overnight dialysis at 5°C. ●, liposomes stored for 21 days at 2–6°C prior to overnight dialysis. D, 2,3-dipalmitoylcyclopentano-1-phosphocholine with four methylene groups between the nitrogen and phosphorus atoms (DPCyPC) [11]. ○, liposomes not stored in the cold prior to overnight dialysis at 5°C (data taken from Ref. 11). ●, liposomes stored for 14 days at 2–6°C prior to overnight dialysis. DSC scans for these four lipids are also included (endotherms upwards). Scans for $C_{16}PC$, $C_{17}PC$ and $C_{18}PC$ are from Ref. 12. The scan for DPCyPC was run by Dr. M.K. Jain (personal communication). The lipid dispersions were stored in the cold for sufficient time to induce subgel phases.



Lipid mixtures

Fig. 2 illustrates the DSC scans for dispersions of $C_{16}PC$, $C_{14}PC$ and an equimolar mixture of $C_{16}PC/C_{14}PC$ which have been stored in the cold for prolonged periods of time. The scans for the main and pre-transitions and for the subtransition in $C_{16}PC$ are in excellent agreement with those of previous workers [4,21] (bearing in mind the usual intra-laboratory instrumental and preparational details.) The scan for $C_{16}PC/C_{14}PC$ shows that subtransitions form in liposomes which are composed of mixtures. Because of the length of time that it takes these subtransitions to appear, we have not yet completed detailed kinetic studies on the formation of the subtransitions; however, subtransitions in $C_{16}PC/C_{14}PC$ mixtures take considerably longer to form than they do in $C_{16}PC$. The scan for $C_{14}PC$ shows a subtransition after 208 days which is hitherto unreported by others [4,21], probably because of sample preparation and/or formation time. The transition enthalpies are main 6.0, pre 1.2 and sub 5.4 kcal/mol, ±10%, and the corresponding peak temperatures, 24.5, 15.8 and 17.7°C. It is of interest that the peak temperature of the pre-transition for $C_{14}PC$ is lower than that of its subtransition, in contradistinction to all previously reported C_nPCs but reminiscent of the situation in the corresponding ethanolamines [8–10]. It is not clear what will be the crystal packing below the subtransition of $C_{14}PC$.

Fig. 3A shows the temperature at the transition

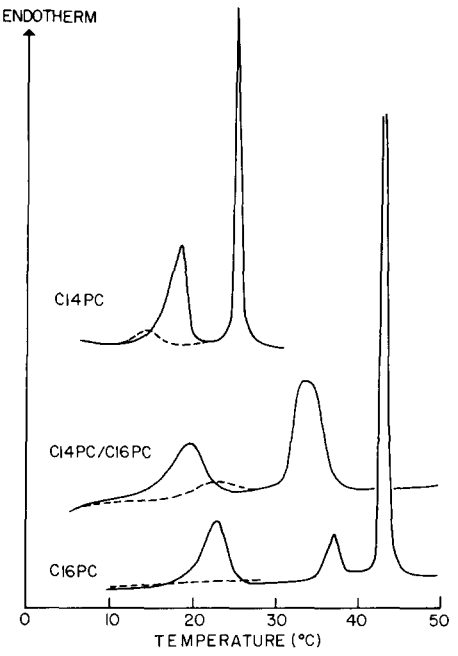


Fig. 2. DSC scans of liposomes of saturated diacylphosphatidylcholines of number of carbons per acyl chain 16 ($C_{16}PC$), an equimolar mixture of $C_{14}PC$ and $C_{16}PC$, and $C_{14}PC$, stored at 2–4°C for 11, 168 and 208 days, respectively. The dotted lines are for a scan done immediately after the solid line scan, so showing the pre-transition. Illustrated are tracings of the chart recorder; an instrumental temperature (time-constant) correction of −0.6 deg. C has yet to be applied.

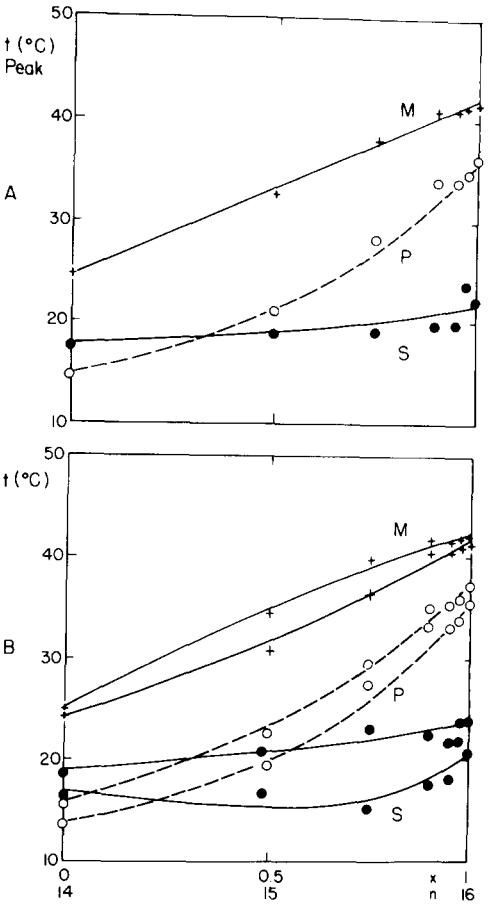


Fig. 3. Results of DSC measurements on mixtures of saturated diacylphosphatidylcholines of $C_{14}PC$ and $C_{16}PC$ for the main (M), pre- (P) and sub- (S) transitions. A, peak temperature of transition versus mole fraction x of $C_{16}PC$. B, phase diagrams for the transition (derived from FWHM – see text). Curves drawn by eye. n is the effective number of carbon atoms per acyl chain.

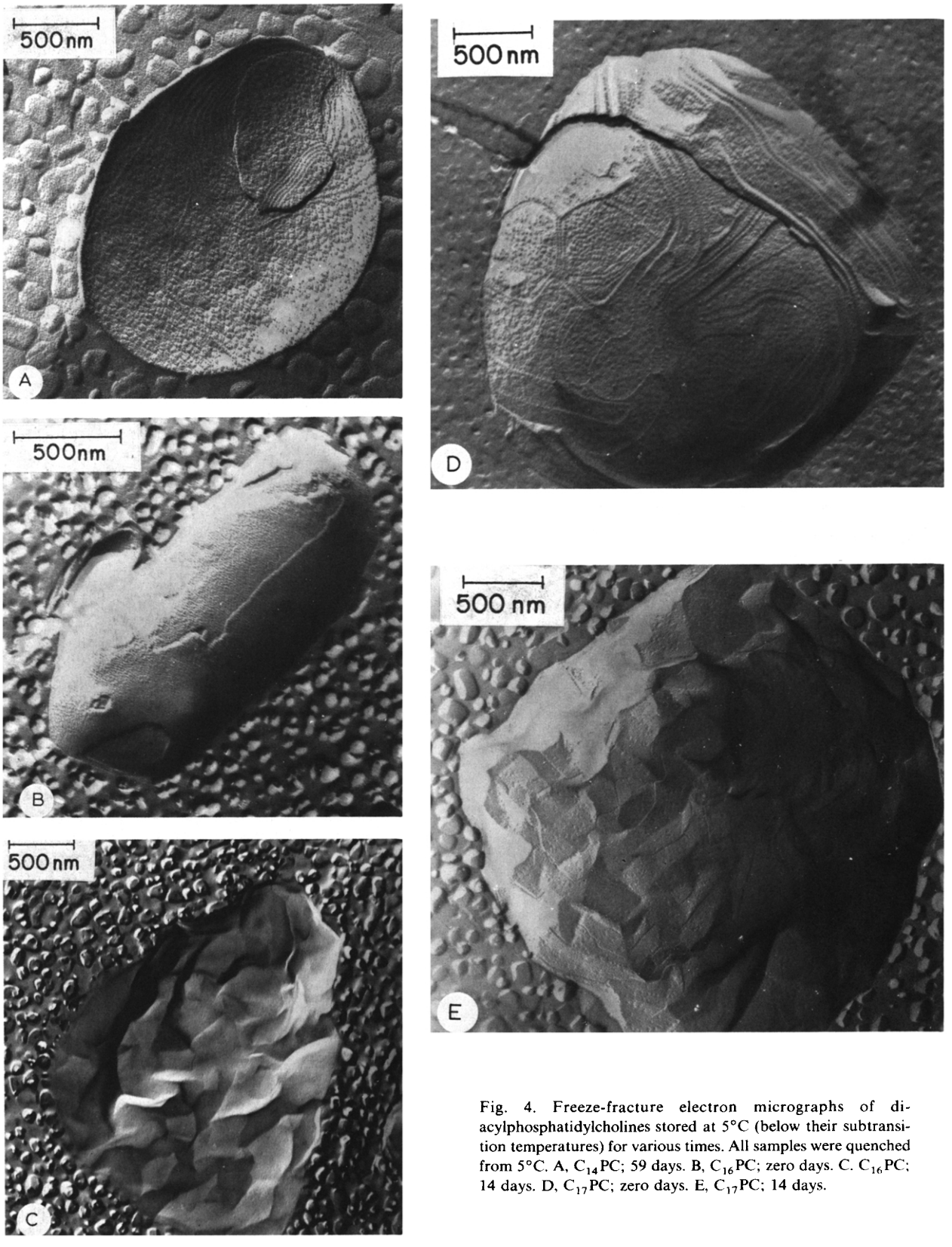


Fig. 4. Freeze-fracture electron micrographs of diacylphosphatidylcholines stored at 5°C (below their subtransition temperatures) for various times. All samples were quenched from 5°C. A, C_{14}PC; 59 days. B, C_{16}PC; zero days. C. C_{16}PC; 14 days. D, C_{17}PC; zero days. E, C_{17}PC; 14 days.

peaks versus chain length n. In agreement with previous studies [22,23] the slopes for the main and pre-transitions are substantial and similar, indicating the dependence of these transitions upon acyl chain length. However, Fig 3A shows that the slope for the subtransition is quite shallow, and hence the subtransition is (almost) independent of acyl chain length. This implies that the subtransition in phosphatidylcholines is predominantly influenced by the head-group, and/or glycerol backbone, a finding which is supported by results obtained with saturated diacyl lipids possessing ethanolamine head-groups [8–10,24] and with phospholipids in which cyclopentanetriol has replaced glycerol [11]. Fig. 3B shows a measure of the phase diagram for $C_{16}PC/C_{14}PC$ mixtures, constructed by superimposing the full width at half maximum (FWHM) of a transition peak onto the curves of Fig. 3A.

Normally, one constructs a phase diagram by using both heating and cooling scans to obtain the solidus and liquidus curves as a function of composition [25,26]. However, since the subtransition is only apparent on the first heating scan and not on subsequent scans [4,21], this technique cannot be used. Thus, the FWHM was chosen as a substitute measure to approximate the onset and completion of a phase change.

Morphology

Fig. 4 displays freeze-fracture electron micrographs of dispersions of $C_{16}PC$ and $C_{17}PC$ stored for various times at 2–6°C. Vesicles quenched below the main phase transition temperature, but without prior storage in the cold, display a smooth surface with some banding in the case of $C_{17}PC$. After storage in the cold for 14 days the surface morphology changes and becomes crinkled in appearance for both $C_{16}PC$ and $C_{17}PC$. This crinkled appearance is very similar to that previously reported [11] for a different cyclopentanetriol analogue of $C_{16}PC$ which exhibits a close packed orthorhombic type of crystalline arrangement below its main transition temperature, even without prior storage in the cold.

The morphology for $C_{14}PC$ left in the cold for even 59 days exhibited no crinkling. Thus, crinkling is not simply an artefact; its absence from $C_{14}PC$ may simply reflect the tardiness of formation of the subtransition.

Discussion

It is now evident that the phase scheme for saturated phospholipids is very complex. For PCs the gel state must be considerered metastable, since a new low temperature phase forms when these lipids are stored in the cold for variable periods of time, dependent upon the acyl chain length. X-ray diffraction measurements indicate that in this new phase, the acyl chains are closely packed in a quasi-orthorhombic arrangement [7]. It has also been suggested [7] that formation of this low temperature phase involves a partial dehydration of the head-group region.

Ion permeability

The phase properties of lipid dispersions are also reflected in their permeability characteristics. When PCs pass through the main gel to liquid crystal transition, a permeability increase is noted, the height of which is inversely related to the acyl chain length. However, permeability measurements should be viewed as a physical technique for monitoring phospholipid membrane structure in which a probe, in this case ^{22}Na, samples the entire thickness of the bilayer across which it passes. Although alterations in transmembrane ion movement do reflect underlying membrane phase changes, there is not a strict correlation between these two. For example, although the main transition endotherm by DSC is usually quite sharp (FWHM 1.0 deg. C), the permeability change is much broader (Fig. 1), indicating that the permeability measurements sense both pre- and post-melt phenomena. Currently, the most plausible explanation is that the permeability peak at the main transition is due to enhanced diffusion across defects resulting from packing mismatches [27].

As illustrated in Fig. 1, baseline permeability (at temperatures below the main transition) decreases in dispersions stored in the cold. This phenomenon is not due to formation of a new phase, since it occurs at temperatures above the subtransition, but must involve some subtle alteration in lipid packing, resulting in annealing of membrane defects. Stored dispersions of $C_{16}PC$, a $C_{16}PC$ analogue and $C_{17}PC$ also display small, but definite permeability maxima at temperatures corresponding to the DSC measured subtransitions.

By analogy with the main transition, it would seem reasonable to postulate that these permeability maxima result from enhanced diffusion across defects where subgel and gel phase lipid interface. These solid-solid packing mismatches clearly do not lead to the large permeability changes of the solid-liquid mismatches occurring at the main phase transition.

C_{18}PC dispersions display two small permeability peaks at 26°C and 36°C which do not correspond to DSC-measured transitions. The relationship between these two permeability peaks and the DSC subtransition in this lipid remains unclear at this time: Possibly the present discrepancy between the two techniques depends on the acyl chain length.

Mixtures

As illustrated in Figs. 2 and 3, mixtures of C_{14}PC/C_{16}PC, even those of equimolar ratio, display a subtransition when stored for a sufficient period of time in the cold. Initially we observed subtransitions only in mixtures containing 5 mol% or less of C_{14}PC [12] but it is now apparent that a larger proportion of C_{14}PC in the mixture decreases the rate of formation of the subgel phase rather than preventing its ultimate appearance.

Even for the pure lipid components, the FWHM of the main transition is well known to be quite finite. Phase diagrams for mixtures of C_{16}PC and C_{14}PC have been constructed for the main transition by calorimetry [25] and by other methods [26]. A lens-shaped diagram results, showing that mixing is close to ideal in the gel and liquid crystal phases. The procedure of using the FWHM duplicates this previous work. Because of the natural temperature broadness of the pre- and subtransitions, it is not so easy to define the onset and end of a transition, so the FWHM is a useful measure. Fig. 3B shows that the phase diagram for the pre- and subtransitions is almost independent of the molar fraction of the components. Hence, for the pre- and subtransitions these results are consistent with ideal mixing, for only a large non-ideality would result in a larger FWHM for the mixtures than is seen in the single-component lipids.

It is curious that of all the C_nPC series which we [12] ($n = 14$, 16, 17, 18 and mixtures of 14/16 and 16/17) and others [4,21] ($n = 15$–18) have examined, subtransitions form fastest, and the main transition is the sharpest, in C_{16}PC. These two properties may be linked: the presence of impurities would be expected to broaden the main transition and to retard (subgel) phase formation. Since the properties of C_nPC lipids are generally a monotonic function of n, it is unlikely that subtransition formation or main peak sharpness are intrinsic to an n of 16. It is possible that the preparative method is optimal for C_{16}PC. This optimization may involve aspects of solvents, temperature, crystallization and starting material.

The continuity of the results for mixtures of lipids shows that mixtures can be used experimentally to interpolate the properties of separate lipids, especially for the subtransitions.

The relative independence of the subtransition on acyl chain length as compared to the main and pre-transitions for the range $n = 14$–16 (Fig. 3A) shows that the subtransition depends predominantly on the non-acyl moiety of the lipid (cf. a tentative conclusion from a restricted range of lipids). As suggested by Ruocco and Shipley [7], the formation of subtransitions involves a change in lipid hydration: Our results are consistent with such hydration changes at the head-group and/or glycerol-backbone region. The phase properties of cyclopentanetriol analogues of C_{16}PC [11] underscore the complex inter-relationship between head-group size and glycerol-backbone structure in the formation of subtransitions. The slow development of the subtransition and the impermeability of the bilayer below this phase transition (Fig. 1) are consistent with a solid state-like phase change.

Morphology

The morphology of vesicles of C_{16}PC and C_{17}PC as visualized by freeze-fracture microscopy (Fig. 4) is of some interest. Vesicles stored in the cold for 14 days demonstrate a crinkled surface analogous to the appearance of the 'high enthalpy' cyclopentanetriol C_{16}PC analogue below its main transition as described previously (Figs. 6d and 6e of Ref. [11]). C_{16}PC, C_{17}PC and this C_{16}PC analogue exist in a closely approximated 'crystal-like' packing under these conditions. C_{14}PC vesicles did not display a crinkled surface after 59 days storage in the cold, consistent with the observation

that the subgel phase for this lipid takes many months to form fully. The explanation for this crinkled appearance is unknown but we offer the following speculation. The mechanical properties of lipid bilayers in the crystal-like packing of the subgel phase must differ from those of the gel or liquid crystal phases. We suggest that bilayers in the more 'rigid' subgel phase crinkle when frozen and fractured as part of the technique of freeze-fracture microscopy, whereas gel or liquid crystal phase bilayers do not behave in a similar fashion. The subgel phase is dehydrated compared to the gel phase [7] and may be expected to be more rigid. In part then, the crinkled appearance could be the result of the interplay between the mechanical properties of the subgel phase and the technique itself of freeze-fracture preparation.

As noted under methods, glycerol is used to reduce ice crystal formation. Although this compound may affect the kinetics of subtransition formation it is certainly not responsible for the morphologic features noted in Fig. 4, since $C_{16}PC$ and $C_{17}PC$ demonstrated 'crinkling' only after 14 days of cold incubation, whereas $C_{14}PC$ did not display this appearance even after 59 days of cold storage. In addition, $C_{16}PC$ dispersions prepared in 35% v/v (to give a freezing-point depression of $-25°C$) ethylene glycol, a more effective cryoprotectant than glycerol, develop a typical subtransition (as measured by DSC) after 14 days storage in the cold. The main and pre-transitions of such dispersions are similar to those prepared in the absence of ethylene glycol. Finally, it should be stressed that we are only suggesting that this 'crinkled' appearance is a morphologic correlate of the subgel phase. To confirm such a correlation, one would have to examine many more lipids stored for variable periods of time in the cold.

In conclusion, for the saturated phosphatidylcholines, the gel phase is metastable, and a new, low temperature, thermodynamically stable subgel phase forms under appropriate conditions. Development of the subgel phase appears (for the range of lipids that we studied) to depend primarily on the glycerol backbone and/or polar head-group region. These changes probably involve a dehydration step [7] such that the phospholipid molecules can pack together in a closely approximated arrangement. The permeability experiments confirm that subgel lipids can form closed vesicles and that such vesicles maintain an effective barriers to ions such as sodium. Furthermore, it appears that the subgel phase can tolerate a mixture of lipids differing in acyl chain length by two carbons without any appreciable disruption in the existing packing arrangement. Freeze-fracture electron microscopy reveals that the surface morphology of the subgel phase differs from that of the gel phase, possibly due to the differing mechanical properties of these two packing arrangements. An ultimate goal is to present a Gibbs free energy diagram versus temperature [10] or acyl chain length for the various lipid phases. The stable phase would be that with the lowest free energy under the conditions examined. Studies on the thermal behaviour of saturated phosphatidylcholines will help unravel the behaviour of more biologically relevant lipids.

Hence, our present working model is that the existence and formation of the subgel phase depend critically upon the interaction of water with the phosphocholine head-group and/or glycerol-backbone region, and relatively little upon the acyl chains.

Acknowledgements

We thank Dr. Anthony Hancock for DPCyPC, Dr. Mahendra K. Jain for the DSC scan of DPCyPC and comments, Dr. John R. Silvius for a helpful conversation, Mr. Man Yat Tse (National Research Council of Canada summer student), Mrs. Marlene Young and Mrs. Maria Dinda for technical assistance, Mrs. Jackie Snider for typing, the Medical Research Council for support to M.A. S., and Queen's University for a Queen's Quest Research Scholar Award to L.F.

References

1 Bangham, A.D., Standish, M.M. and Watkins, J.C. (1965) J. Mol. Biol. 13, 238–252
2 Bangham, A.D. (1981) in Research Monographs in Cell and Tissue Physiology, Vol. 7, Liposomes: From physical structure to therapeutic applications. (Knight, C.G., ed.), pp. 1–17, Elsevier/North Holland, Amsterdam
3 Juliano, R.L. (1981) in Research Monographs in Cell and Tissue Physiology, Vol. 7, Liposomes: From physical structure to therapeutic applications. (Knight, C.G., ed.), pp. 391–407, Elsevier/North Holland, Amsterdam

4 Chen, S.C. Sturtevant, J.M. and Gaffney, B. (1980) Proc. Natl. Acad. Sci. USA 77, 5060–5063
5 Nagle, J.F. and Wilkinson, D.A. (1982) Biochemistry 16 3817–3821
6 Fuldner, H.H. (1981) Biochemistry 20, 5707–5710
7 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 684, 59–66
8 Chang, H. and Epand, R.M. (1983) Biochim. Biophys. Acta 728, 319–324
9 Seddon, J.M., Harlos, K. and Marsh, D. (1983) J. Biol. Chem. 258, 3850–3854
10 Wilkinson, D.A. and Nagle, J.F. (1984) Biochemistry 23, 1538–1541
11 Jain, M.K., Singer, M.A., Crecely, R.W., Pajouhesh, H. and Hancock, A.J. (1984) Biochim. Biophys. Acta 774, 199–205
12 Finegold, L. and Singer, M.A. (1984) Chem. Phys. Lipids 35, 291–297
13 Singer, M.A. (1982) Chem. Phys. Lipids 31, 145–159
14 Van Dooren, A.A. and Muller, B.W. (1981) Thermochim. Acta 49, 151–184
15 Finegold, L. and Kumar, P.K. (1980) Biopolymers 19, 1567–1569
16 Kumar, P.K. and Finegold, L. (1981) Thermochim. Acta 48, 51–59
17 Singer, M. (1981) Chem. Phys. Lipids 28, 253–267
18 Papahadjopoulos, D. and Watkins, J.C. (1967) Biochim. Biophys. Acta 135, 638–652
19 Szoka, F., Jr. and Paphadjopoulos, D. (1980) Annu. Rev. Biophys. Bioeng. 9, 467–508
20 Bangham, A.D., De Gier, J. and Greville, G.D. (1967) Chem. Phys. Lipids 1, 225–246
21 Stumpel, J., Eibl, H. and Nicksch, A. (1983) Biochim. Biophys. Acta 727, 246–254
22 Silvius, J.R., Read, B.D. and McElhaney, R.N. (1979) Biochim. Biophys. Acta 555, 175–178
23 Silvius, J.R. (1982) in Lipid-Protein Interactions (Jost, P. and Griffith, O.H., eds.), pp. 239–281, Wiley, New York
24 Mulukutla, S. and Shipley, G.G. (1984) Biochemistry 23, 2514–2519
25 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. USA 73, 3862–3866
26 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 285–344
27 Jain, M.K. (1983) in Fluidity in Biology (Aloia, R.C., ed.) pp. 1–37, Academic Press, New York