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LIPID PHASE TRANSITIONS IN MODEL BIOMEMBRANES

THE EFFECT OF IONS ON PHOSPHATIDYLCHOLINE BILAYERS

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Summary

Differential scanning calorimetry has been used to study the endothermic phase behaviour of some model biomembranes (i.e. phosphatidylcholine-water systems) in the presence of a wide range of alkaline, alkaline earth and heavy metal salts. Studies and comparisons were made of both cation and anion effects. Shifts occur in the temperatures of both the pre-transition and main transition endotherms. The observed shifts are smaller than those which have been reported for charged lipids, and no evidence has been found for the formation of specific complexes. Electron microscopic studies on freeze-fractured dispersions of phosphatidylcholine-water-salt systems show that with some salts the typical rippled surface observed with L-α-dimyristoyl phosphatidylcholine, when in the gel state, is replaced by a smooth surface.

Introduction

Previous studies of pure lipids and lipid-water systems using infrared spectroscopy [1], differential thermal analysis, differential scanning calorimetry [2—5] and NMR spectroscopy [6] showed the existence of a thermotropic phase transition associated with hydrocarbon chain melting involving increased rotational and translational motions of these chains. The temperatures at which these endothermic phase transitions occur have been shown to be dependent upon the head group, the alkyl chain length and the degree and type of unsaturation present [7]. The presence of a third component such as cholesterol [8,9], proteins [10—12], drugs [13—15] and salts [11,16] can cause significant shifts in the phase transition temperature or eliminate the phase transition. Interest in these systems is great because of their relevance to biomembrane systems.

In this paper we examine the effects of various cations and anions on the endothermic phase transitions of two phosphatidylcholine-water systems. Previous studies of ion effects have been reported [11,16] but are of a less extensive nature than those reported here.

Materials and Methods

The lipids, L-α-dimyristoyl phosphatidylcholine and L-α-dipalmitoyl phosphatidylcholine used in the calorimetric experiments were obtained from Koch-Light Laboratories Ltd. The dimyristoyl phosphatidylcholine used in the electron microscopic studies was obtained from Fluka. Purity was checked by thin-layer chromatography. The salts used were usually of Analar quality but in some cases reagent grade was used. The solutions were prepared volumetrically using deionised distilled water. Samples were prepared by homogenising weighed amounts of the appropriate lipid and solution above the gel-liquid crystalline transition temperature (approx. 50°C) using a vortex mixer. Some samples were homogenised by centrifuging them backwards and forwards through a constriction in the sample tube. The different methods of sample preparation did not significantly alter the results obtained.

Calorimetric measurements were performed using a Perkin-Elmer DSC-1B on samples weighing approx. 8 mg contained in hermetically sealed aluminium pans with a heating rate of 8K/min. Some of the results were checked using a Perkin-Elmer DSC-2 with a heating rate of 5 K/min, no significant difference being obtained in the results from the two instruments. The temperature scale was calibrated using the transition temperatures of cyclohexane (279.8 K), sodium sulphate decahydrate (305.6 K) and indium (429.8 K), and was accurate to ±0.2 K. The indium transition was also used to calibrate the enthalpy per unit area. A minimum of three different samples were prepared for each system studied and each sample was investigated at least twice. The extrapolated onset temperature was taken as the transition temperature. Enthalpy and entropy data for the observed transitions were calculated from the area below the endotherms by standard methods, the curve areas being measured with a fixed arm planimeter.

Dispersions of dimyristoyl phosphatidylcholine in excess solution (usually approx. 1 : 2 or 1 : 3 by weight), homogenised as described above, were quenched from 15°C into liquid Freon at −80°C and then liquid nitrogen. Fracturing and etching, using a Nanotech freeze-fracture apparatus, were carried out at −99°C following by Pt/C shadowing and carbon replication at −110°C.

Results

Fig. 1 shows the calorimetric curves for the dimyristoyl phosphatidylcholine-water system as a function of water content. The endotherm associated with the ice-melting transition at approx. 0°C has been omitted from the diagrams. At first only one transition occurs but with increasing water content this transition narrows and a second transition appears slightly lower in temperature. This has been called the pre-transition endotherm, the other being the

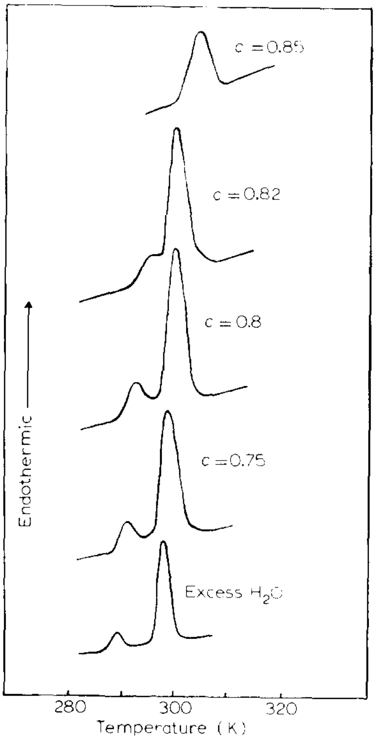

Fig. 1. Calorimetric curves for the dimyristoyl phosphatidylcholine-water system as a function of lipid concentration, c = wt. lipid/wt. lipid + wt. water.

main transition endotherm. The characteristic behaviour of these features with changing water content has been reported elsewhere [5]. There is an abrupt change in the temperatures at which the two transitions occur at weight fractions of lipid of approx. 0.8. This also coincides with the appearance of the ice-melting endotherm in the calorimetric curves. For the dipalmitoyl phosphatidylcholine-water system this change is approximately paralleled by changes in the enthalpy associated with the main transition (Table I). In view of this marked dependence of transition temperature on the water content, the studies involving ionic solutions were performed at concentrations well removed from this critical region, a 1 : 1 by weight lipid/solution mixture being used.

Table II shows the dependence of the pre and main transition temperatures (T_p and T_c) of the dipalmitoyl phosphatidylcholine-water system on the presence of some heavy metal salts at low concentrations. It is apparent that salt concentrations as low as 10^{-3} M are inducing perturbations in the phase transitions, with a given salt affecting the two lipid transitions in an approximately similar fashion.

Table III illustrates the effect of some heavy metal salts at concentrations up to 1 M on the transition temperatures of dimyristoyl phosphatidylcholine-water dispersions. All of the salts investigated increased the transition temperature of both endotherms but it is apparent that the efficacy of the various salts differs. The very large effects of $ZnCl_2$ and $SnCl_2$ are particularly notable and it is also significant that in the systems containing these salts the pre-transition appeared as a shoulder on the main transition endotherm.

TABLE I

THE ENTHALPY (ΔH) AND ENTROPY (ΔS) CHANGES ASSOCIATED WITH THE PRE- (p) AND MAIN (c) PHASE TRANSITIONS FOR THE DIPALMITOYL PHOSPHATIDYLCHOLINE-WATER SYSTEM AS A FUNCTION OF THE WEIGHT FRACTION OF THE LIPID

Weight fraction of dipalmitoyl phosphatidylcholine (C)	ΔH_p (kcal · mol^{-1})	ΔS_p (cal · K^{-1} · mol^{-1})	ΔH_c (kcal · mol^{-1})	ΔS_c (cal · mol^{-1} · K^{-1})
0.99	—	—	5.9	17.7
0.833	—	—	6.5	20.0
0.768	—	—	8.6	28.0
0.714	—	—	9.5	31.0
0.666	1.3	4.3	9.4	30.4
0.625	1.6	5.3	9.1	29.4
0.5	1.7	5.5	9.5	32.8
Excess water			8.7	27.6 *
Excess water			9.7	30.7 **

* Ref. 32.
** Ref. 33.

TABLE II

THE PRE-TRANSITION ENDOTHERM AND MAIN ENDOTHERM TRANSITION TEMPERATURES OF DIPALMITOYL PHOSPHATIDYLCHOLINE-HEAVY METAL SALT SOLUTION SYSTEMS (C = 0.5)

Solution	T_p (K)	T_c (K)
Water	308.5	314.5
10^{-3} M $HgCl_2$	309.9	316.7
10^{-3} M $ZnCl_2$	309.7	315.6
10^{-3} M $SnCl_2$	307.9	314.5
10^{-3} M $CuCl_2$	308.5	314.0
10^{-3} M $CdCl_2$	308.2	314.0
10^{-3} M lead acetate	309.4	316.5
10^{-3} M $CuSO_4$	307.8	314.0

TABLE III

THE PRE-TRANSITION ENDOTHERM AND MAIN ENDOTHERM TRANSITION TEMPERATURES OF DIMYRISTOYL PHOSPHATIDYLCHOLINE—HEAVY METAL SALT SOLUTION SYSTEMS (C = 0.5)

Solution	T_p (K)	T_c (K)
Water	287.2	296.7
0.1 M $ZnCl_2$	—	302.4
0.1 M $SnCl_2$	—	301.2
1 M $CdCl_2$	290.0	297.7
1 M $AgNO_3$	290.4	298.7
1 M lead acetate	287.7	297.7
1 M $CuSO_4$	291.4	297.9
1 M thallium acetate	287.3	296.9

TABLE IV

THE PRE—TRANSITION ENDOTHERM AND MAIN ENDOTHERM TRANSITION TEMPERATURES OF DIMYRISTOYL PHOSPHATIDYLCHOLINE-ALKALINE, ALKALINE EARTH AND GUANADINIUM (Gu) SALT SOLUTION SYSTEMS (C = 0.5)

Solution	T_p (K)	T_c (K)
1 M LiCl	287.2	295.9
1 M NaCl	289.2	297.8
1 M KCl	288.6	297.4
1 M RbCl	288.2	297.6
1 M CsCl	286.7	297.0
1 M NaF	289.9	298.2
1 M NaBr	284.1	296.7
1 M NaI	—	294.3
1 M NaCNO	287.6	297.2
1 M sodium acetate	290.6	297.6
1 M KCN	287.0	297.2
1 M NH_4Cl	290.2	299.3
1 M tetramethylammonium chloride	289.2	297.4
1 M $CaCl_2$	—	301.7
1 M $MgCl_2$	292.7	298.8
1 M GuSCN	—	292.9
0.1 M GuCl	287.1	296.7
0.2 M GuCl	286.1	296.6
1 M GuCl	281.3	296.3

Table IV illustrates the effects of a comprehensive range of salts on the transition temperatures of the dimyristoyl phosphatidylcholine-water system. For 1 M $CaCl_2$, NaSCN and GuSCN no pre-transition endotherms were observable, and for 1 M NaI this endotherm was so broad that it was not possible to obtain a reliable estimate of the transition temperature. Table V gives the entropy and enthalpy values associated with the phase transitions for some of the salts studied.

TABLE V

ΔH AND ΔS DATA FOR DIMYRISTOYL PHOSPHATIDYLCHOLINE/AQUEOUS SALT SOLUTIONS

Estimated errors: $\Delta H_c \pm 0.9$ kcal · mol^{-1}; $\Delta S_c \pm 2$ cal · mol^{-1} · K^{-1}.

Solution	ΔH_p (kcal · mol^{-1})	ΔS_p (cal · mol^{-1} · K^{-1})	ΔH_c (kcal · mol^{-1})	ΔS_c (cal · mol^{-1} · K^{-1})
Water	—	—	6.7	22.4 *
1 M NH_4Cl	1.2	4.1	7.2	23.7
1 M tetramethyl-ammonium chloride	0.8	2.1	6.6	22.4
1 M sodium acetate	1.0	3.6	6.5	22.1
1 M NaBr	0.8	2.7	7.4	25.0
1 M NaCNO	0.7	2.5	6.8	22.9
1 M NaF	0.9	3.2	6.8	23.0
1 M NaI	—	—	6.9	23.8
1 M KCN	0.7	2.6	7.1	24.1
0.1 M $ZnCl_2$	0.7	2.4	7.2	24.5
1 M $MgCl_2$	1.2	4.0	7.6	25.5
1 M $CaCl_2$	—	—	8.6	28.8 **

* Ref. 32.

** The large values of ΔH_c and ΔS_c produced by this salt are probably due to some contribution from a pre-transition coincident with the main endotherm.

Figs. 2a and 2b show the marked effect of various NaSCN concentrations on the dimyristoyl phosphatidylcholine-water system at 50 weight percent lipid. At SCN^- concentrations >0.2 M no pre-transition endotherm is observable and indeed as the concentration of salt approaches this value the endotherm is broadening. The main transition temperature increases rapidly at low concentrations and thereafter increases more slowly. The most significant effect however, is the way in which the endotherms diverge as the salt concentration increases.

In view of the importance of possible hydration effects the dipalmitoyl phosphatidylcholine-water-$ZnCl_2$ system was studied in more detail. The mol ratio of dipalmitoyl phosphatidylcholine/$ZnCl_2$ was kept constant at 3 : 1 and the water content of the system was varied. The results for the main transition endotherm are shown in Fig. 3. It can be seen that $ZnCl_2$ modifies the effect

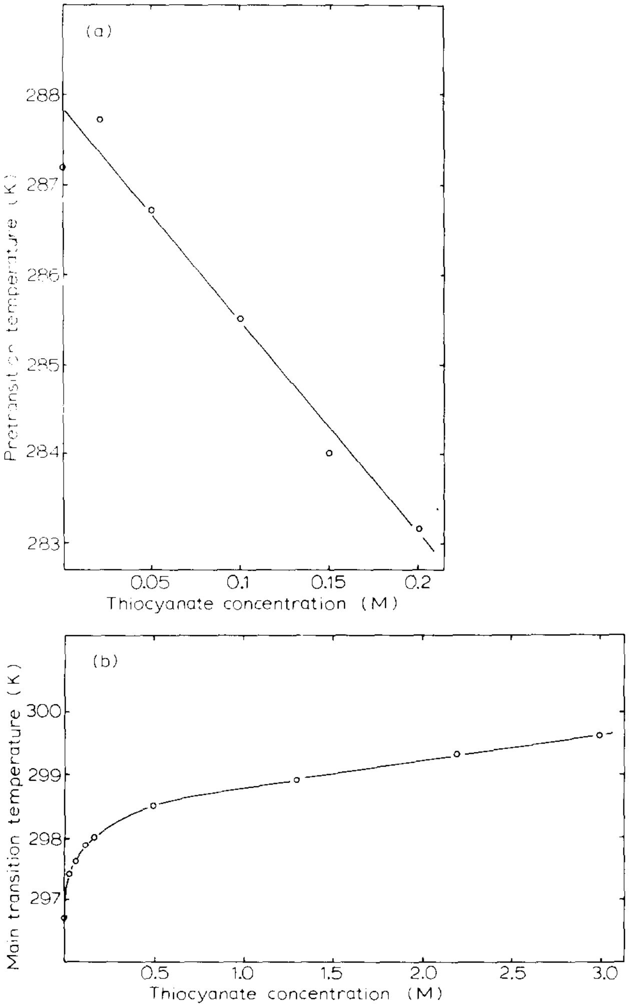

Fig. 2. The dependence of (a) the pre-transition endotherm and (b) the main endotherm transition temperatures of dimyristoyl phosphatidylcholine on NaSCN concentration. $C = 0.5$.

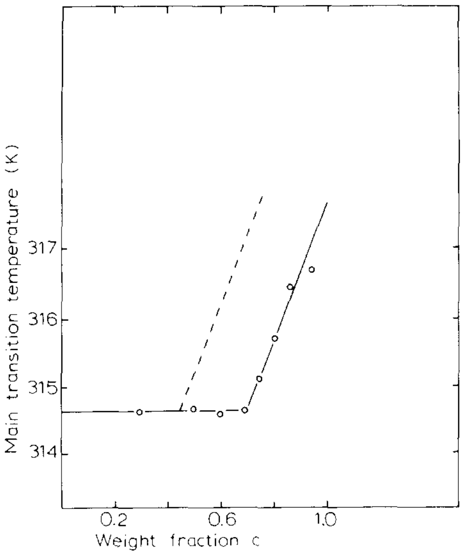

Fig. 3. The dependence of the main endotherm transition temperature on water concentration for the dipalmitoyl phosphatidylcholine-water (full line) and dipalmitoyl phosphatidylcholine-water-$ZnCl_2$ (dashed line) systems. Ratio dipalmitoyl phosphatidylcholine/Zn^{2+} was 3 : 1 throughout.

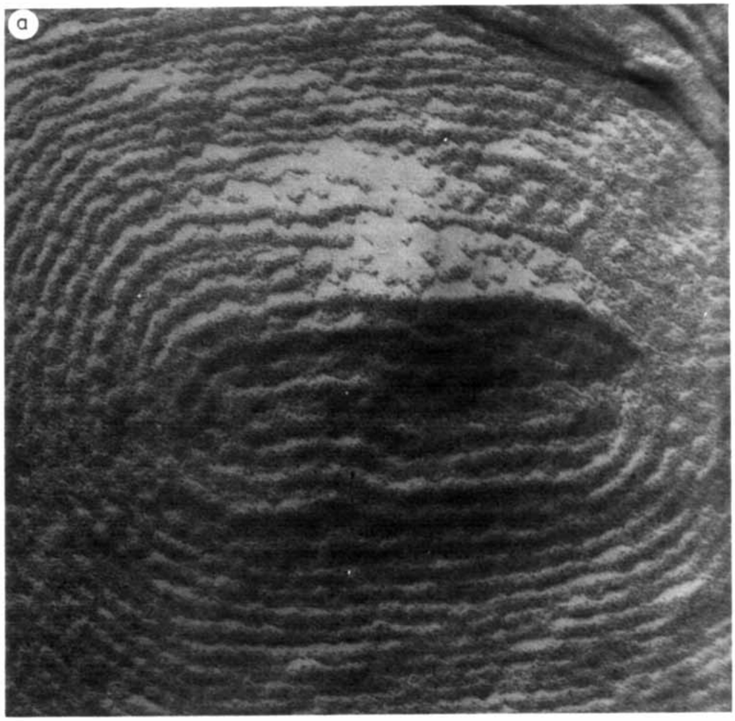

Fig. 4. For legend see opposite page.

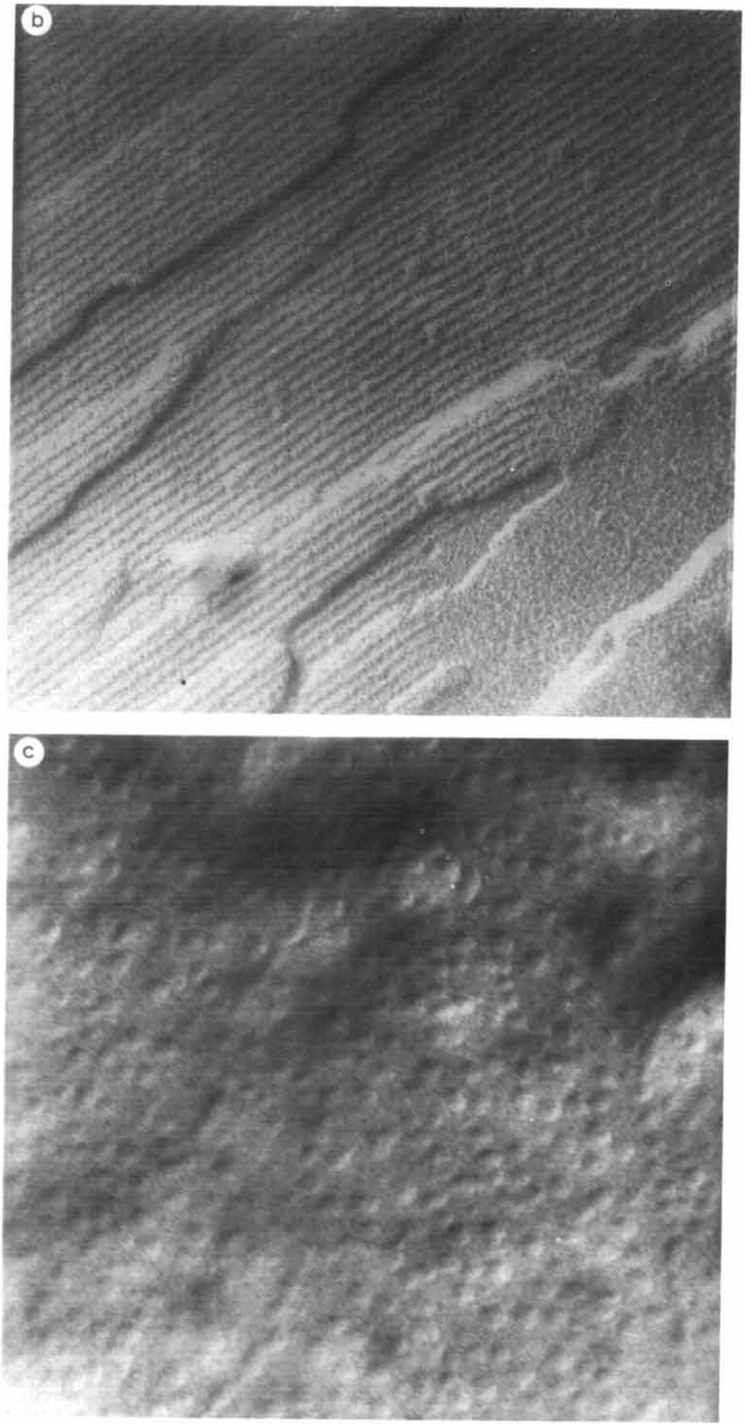

Fig. 4. Freeze-fracture surfaces: a, dimyristoyl phosphatidylcholine-water; b, dimyristoyl phosphatidyl choline-1 M GuCl; c, dimyristoyl phosphatidylcholine-1 M NaSCN. $C = 0.25$. Magnification × 50 000. (At these concentrations GuCl does not remove the pre-transition whilst NaSCN does remove this transition).

of water on dipalmitoyl phosphatidylcholine since the abrupt change in transition temperature has shifted from 30% water to 55% water by weight. However, there was no significant alteration in the heat absorbed during the transition.

Electron microscopic studies on the freeze-fractured surfaces of dispersions of dimyristoyl phosphatidylcholine with various salt solutions have shown that in cases where the pre-transition endotherm has been removed, the ripples usually observed on the surfaces of the liposomes have also been removed. Electron micrographs of the different surfaces observed are shown in Fig. 4.

Discussion

Previous results on the interaction of metal ions with charged and uncharged lipids using a variety of physical techniques have been interpreted using double layer theory [17,18], dehydration effects [19], complex formation [11,16, 20—24] and structure making and breaking effects [21,25]. It is to be expected that the interaction of metal ions with uncharged phosphatidylcholine should produce only small effects and the work of Simon et al. [16] and the results reported here show this is so. The transition temperatures are shifted by 6°C at the most and the enthalpy change associated with the transition is hardly affected by the presence of salt (Table V).

The behaviour observed experimentally of the effects of salts on the transition temperatures in phosphatidylcholine-water systems is apparently rather complex. Thermodynamic arguments developed in the appendix give an insight into the sort of behaviour we would expect for the effect of solutes on a phase transition. It is shown that the temperature change in a phase transition depends on four unknown parameters for a given salt. In view of this any precise and unambigous interpretation of the effect of salts on phase transitions is not possible at present and all one can hope for is some correlation with the experimentally observed effects and observations on some simpler systems. Eqns. 17 and 18 (Appendix) indicate that both anions and cations may affect phase transitions and it is convenient to consider first the order of cation effects by investigating salts with a common anion. The experimental results on systems of metal chlorides show that the temperature of the main transition in the dimyristoyl phosphatidylcholine-water system is in the order (NMe_4^+, tetramethylammonium ion)

$NH_4^+ > Na^+ \approx K^+ \approx Rb^+ \approx NMe_4^+ > Cs^+ > Gu^+ > Li^+$

for monovalent cations and

$Zn^{2+} > Sn^{2+} > Ca^{2+} > Mg^{2+} > Cd^{2+}$

for divalent cations. The order observed for the pre-transition endotherm is

$NH_4^+ > Na^+ \approx NMe_4^+ > K^+ > Rb^+ > Li^+ > Cs^+ > Gu^+$

for the monovalent ions. No pre-transition endotherms were observed for $ZnCl_2$, $SnCl_2$ and $CaCl_2$ whilst the order for $MgCl_2$ and $CdCl_2$ was $Mg^{2+} > Cd^{2+}$. It seems likely that for the first three salts the pre-transition endotherm has been shifted so high in temperature that it is overlapping the main transition.

The dipalmitoyl phosphatidylcholine-water system shows that in the presence of divalent metal chlorides the main transition endotherm decreases in the order

$Hg^{2+} > Zn^{2+} > Sn^{2+} > Cu^{2+} > Cd^{2+}$

and the pre-transition temperatures follow the order

$Hg^{2+} > Zn^{2+} > Cu^{2+} > Cd^{2+} > Sn^{2+}$

Therefore for a given phosphatidylcholine-water-metal chloride system the order for both the main and pre-transition endotherms is the same. There is a rough correlation between the orders observed and the magnitudes of the association constants of metal ions with simple phosphates. The association constants experimentally observed [26,27] vary with the phosphate used but for monovalent cations the order is

$Li^{+} > Na^{+} > K^{+} > Cs^{+}$

and for divalent cations

$Hg^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+} > Ca^{2+} > Mg^{2+}$

The agreement is only approximate and we therefore consider other possible interaction mechanisms.

Some aspects of previously reported lipid-metal ion interactions have been interpreted on the basis of the known structure making and breaking effects of the ions on water [21,25]. Attempts to correlate our wider range of cations with phosphatidylcholine in a similar manner were unsuccessful. Indeed the effect of urea, at a concentration of 1 M, on the transition temperatures was virtually negligible although it is a well-known structure breaker.

In view of the fact that all ions in solution are solvated it is necessary to take account of possible hydration effects in interpreting the results reported here, and the results depicted in Fig. 3 confirm this. It is noteworthy that for salts with a common anion the ratio $(\text{charge})^2/\text{radius}$, (an approximate measure of the solvation interaction), predicts fairly accurately the relative orders of magnitude of the effects produced (Table VI) with the above ratio increasing with the ability of the metal ion to raise the transition temperatures. However, the positions of Li^{+} and Hg^{2+} are anomalous.

It should be noted also that the anionic effect is by no means small and considering the main transition endotherm with sodium salts the temperature of the transition decreases as follows

$SCN^{-} > F^{-} > Cl^{-} \approx \text{acetate}^{-} > CNO^{-} > Br^{-} > I^{-}$

whereas the order observed for the pre-transition is

$\text{acetate}^{-} > F^{-} > Cl^{-} > CNO^{-} > Br^{-} > SCN^{-}$

There is a reasonable correlation in these two series except for the extreme change of position of SCN^{-}. The effectiveness of anions in destroying the structure of water and lowering the hydrogen bond strength is given by ref. 28

$I^{-} > SCN^{-} > Br^{-} > Cl^{-} \approx CNO^{-} > F^{-}$

TABLE VI

RATIO OF $(CHARGE)^2$/IONIC RADIUS FOR METAL IONS STUDIED

Data from 53rd edition Handbook of Chemistry and Physics.

Ion	Radius (Å)	$(\text{Charge})^2$/Radius ($Å^{-1}$)
Cu^{2+}	0.96	4.17
Ag^{+}	1.26	0.794
Zn^{2+}	0.74	5.42
Cd^{2+}	0.97	4.13
Hg^{2+}	1.1	3.64
Tl^{+}	1.49	0.67
Sn^{2+}	0.84	4.76
Pb^{2+}	1.21	3.31
Li^{+}	0.68	1.47
Na^{+}	0.97	1.03
K^{+}	1.33	0.753
Rb^{+}	1.47	0.68
Cs^{+}	1.67	0.6
Mg^{2+}	0.66	6.06
Ca^{2+}	0.99	4.04

There is a correlation between the structure-breaking efficiency of the anions and their ability to lower the transition temperatures. This may be explained by the anions interfering with the hydrogen-bonded structure around the polar head group and hence altering the packing of the alkyl chains.

The very large lowering of the pre-transition temperature in the case of SCN^- may be due to the fact that this ion is known to be adsorbed preferentially at interfaces [29] and therefore may be producing a negatively charged lipid-water interface. This could lead to both longitudinal and lateral repulsion in the bilayer structure, the latter tending to alter the packing of the alkyl chains so leading to a lowering of the pre-transition temperature. Preliminary X-ray measurements have indicated that the long spacings are increasing with increasing SCN^- concentration.

It is difficult to explain the observed increase of the main endotherm transition with increasing SCN^- concentration. It may be that the reorientation of the head group at the pre-transition temperature allows the formation of a new more stable hydrogen bond network, involving SCN^-. If the "melting" and lateral separation of the alkyl chains at the main transition leads to a disruption of such a hydrogen bond network additional thermal energy will be required and so T_c will be raised provided there is no significant change in ΔS_c. This idea of additional hydrogen bonds has been used by Simon et al. [16] to explain the increase in ΔH_c observed for dipalmitoyl phosphatidylcholine + 1 M KSCN.

Simon et al. [16] also found that the main transition temperature decreased for dipalmitoyl phosphatidylcholine + 1 M KSCN. In view of the fact that the temperature changes are small and different chain length lipids and cations were used we consider that the discrepancy is not significant.

The pre-transition phase change is associated with an increase in the mobility of the polar head group and recent work has shown that the transition is also associated with a change in the angle of tilt of the alkyl chains [30]. A simultaneous reduction in the area per polar head group at the lipid-water interface occurs.

Replicas of freeze-fractured surfaces of dimyristoyl phosphatidylcholine-water dispersions in the gel phase, which Luzzati terms $P_{B'}$ [31], have shown rippled surfaces under the electron microscope (Fig. 4). The ripples are thought to be due to undulations in the surface of the liposomes caused by the longitudinal displacement required in packing the tilted chains. Our calorimetric and electron microscopic results support the idea that the pre-transition is associated with the presence of tilted chains, since dispersions of dimyristoyl phosphatidylcholine-water containing salts which remove the pre-transition endotherm show no ripples in their freeze-fractured surfaces. This indicates that the chain tilt, which is a requirement for the presence of ripples, has been altered by the presence of those salts which remove the pre-transition endotherm. This also occurs upon addition of 7% decane which gives a vertical-chain phase.

Conclusions

The effects generally observed are smaller than those which have been observed for charged lipids. The effects of cations appears to correlate approximately with a ratio, (charge)^2/radius, which measures their solvation interaction. Notwithstanding the difficulty of quantitative interpretations some qualitative conclusions can be drawn about the effects of cations on phosphatidylcholine-water systems. It would seem that Hg^{2+}, Pb^{2+} and Zn^{2+} confer increased stability to thermal change on gel phase dispersions of dipalmitoyl phosphatidylcholine whereas Cd^{2+} and Cu^{2+} have a small destabilising effect. With the dimyristoyl phosphatocylcholine-water system it is found that Sn^{2+} and Zn^{2+} have a very marked effect in increasing gel phase stability whereas other heavy metals have rather less effect. The heavy metal salts increase gel phase stability.

Of the common alkaline and alkaline earth salts it may be concluded that Ca^{2+} and Mg^{2+} give rise to increased stability as do Na^+ and K^+ but to a lesser extent.

Anionic effects can be explained qualitatively in terms of structure-breaking effects on the organisation of the polar head group region, leading to an alteration in the packing of the alkyl chains. The anomalous effects of the SCN^- are thought to be due to its great tendency to accumulate at the polar lipid-water interface.

Appendix

We shall consider the behaviour we could expect for the effects of solutes on a phase transition.

Suppose that we consider a phase transition for a mono disperse system

$$\alpha \rightarrow \beta \qquad (1)$$

where α and β are the forms which are stable at low and high temperatures, respectively. At any temperature we have

$$\mu_\alpha(T) = \mu_\beta(T) \tag{2}$$

where $\mu_i(T)$ is the chemical potential of i at temperature T. If we consider the situation when water is the only other species present then expanding chemical potentials and rearranging gives

$$K^{\mathrm{w}}(T) = \frac{f_\beta^{\mathrm{w}}(T)}{f_\alpha^{\mathrm{w}}(T)} = \exp - \left[\frac{\mu_\beta^{\ominus,\mathrm{w}}(T) - \mu_\alpha^{\ominus,\mathrm{w}}(T)}{RT}\right] \tag{3}$$

where $f_\beta^{\mathrm{w}}(T)$ and $f_\alpha^{\mathrm{w}}(T)$ are the fractions of β and α present at temperature T, respectively, $K^{\mathrm{w}}(T)$ is the equilibrium constant for the equilibrium and $\mu_i^{\ominus,\mathrm{w}}$ denotes the standard chemical potential of i in water. Application of the Gibbs-Helmholtz equation gives

$$\left(\frac{\partial \ln K^{\mathrm{w}}(T)}{\partial (1/T)}\right)_p = -\frac{\Delta H^{\ominus,\mathrm{w}}(T)}{R} \tag{4}$$

where $\Delta H^{\ominus,\mathrm{w}}(T)$ is the standard enthalpy change associated with the transition $\alpha \rightarrow \beta$ at temperature T.

If some solute is present as well as the water we may write by analogy with Eqns. 3 and 4

$$K^{\mathrm{s}}(T) = \frac{f_\beta^{\mathrm{s}}(T)}{f_\alpha^{\mathrm{s}}(T)} = \exp - \left[\frac{\mu_\beta^{\ominus,\mathrm{s}}(T) - \mu_\alpha^{\ominus,\mathrm{s}}(T)}{RT}\right] \tag{5}$$

$$\left(\frac{\partial \ln K^{\mathrm{s}}(T)}{\partial (1/T)}\right)_p = -\frac{\Delta H^{\ominus,\mathrm{s}}(T)}{R} \tag{6}$$

The terminology in Eqns. 5 and 6 is such that the superscript "s" denotes that solution is present. When the equilibrium constants $K^{\mathrm{w}}(T)$ and $K^{\mathrm{s}}(T)$ are unity then the corresponding transition temperatures are T_{w} and T_s and we have from Eqns. 3 and 5

$$\frac{\mu_\alpha^{\ominus,\mathrm{w}}(T_{\mathrm{w}}) - \mu_\beta^{\ominus,\mathrm{w}}(T_{\mathrm{w}})}{T_{\mathrm{w}}} = \frac{\mu_\alpha^{\ominus,\mathrm{s}}(T_s) - \mu_\beta^{\ominus,\mathrm{s}}(T_s)}{T_s} \tag{7}$$

and neglecting the temperature change of the heat capacity associated with the phase transitions using Eqn. 6 gives

$$\left[\frac{\mu_\alpha^{\ominus,\mathrm{w}}(T_s) - \mu_\beta^{\ominus,\mathrm{w}}(T_s)}{T_s}\right] - \left[\frac{\mu_\alpha^{\ominus,\mathrm{s}}(T_s) - \mu_\beta^{\ominus,\mathrm{s}}(T_s)}{T_s}\right] = -\left[\frac{1}{T_s} - \frac{1}{T_{\mathrm{w}}}\right] \Delta H^{\ominus,\mathrm{w}}(T_{\mathrm{w}}) \tag{8}$$

If we write

$$\mu_i^{\ominus,\mathrm{s}}(T_s) - \mu_i^{\ominus,\mathrm{w}}(T_s) = \Delta\mu_i^{\ominus,\mathrm{tr}}(T_s) \tag{9}$$

for an i'th species with $\Delta\mu_i^{\ominus,\mathrm{tr}}(T_s)$ being the standard free energy of transfer of species i in its standard states from water to solution, then Eqn. 8 becomes

$$\Delta\mu_\beta^{\ominus,\mathrm{tr}}(T_s) - \Delta\mu_\alpha^{\ominus,\mathrm{tr}}(T_s) = -T_s\left[\frac{1}{T_s} - \frac{1}{T_{\mathrm{w}}}\right] \Delta H^{\ominus,\mathrm{w}}(T_{\mathrm{w}}) \tag{10}$$

It is found experimentally for small molecules at not too high concentrations of solute and can be shown to be generally true for any disperse system that

$$\frac{\Delta\mu_i^{\ominus,\mathrm{tr}}(T)}{RT} = (k_{i-s}c_s) \qquad (11)$$

where k_{i-s} is a constant which depends upon the nature of i and s and c_s is the molar concentration of solute s. The constant k_{i-s} is strictly speaking temperature dependent although investigations on simple systems indicate that the temperature dependence is small and as an approximation we shall assume that it is indeed constant. Consequently from Eqns. 10 and 11 writing $\Delta T = T_\mathrm{w} - T_s$ and $T_s T_\mathrm{w} \cong T_\mathrm{w}^2$ we get

$$\Delta T = \left[\frac{-RT_\mathrm{w}^2}{\Delta H^{\ominus,\mathrm{w}}(T_\mathrm{w})}\right] (k_{\beta-s} - k_{\alpha-s})c_s \qquad (12)$$

Thus at low concentrations of solute we should expect a linear variation of the transition temperature associated with the $\alpha \rightarrow \beta$ phase change with concentration of added solute. The proportionality constant depends upon two terms. The first of these is the usual sort of term obtained for colligative properties and is calculable. The second is a composite term and depends upon the difference between the extent of the interaction of the solute with the α and β forms participating in the general equilibrium. It should be noted that it is not necessary to assume that the solute S actually binds in an associative way to α and/or β. The k_{i-s} terms allow for the fact that rather longer range perturbations of the solute on α and β can exist than would be considered in an association treatment. However, if one wishes to use a chemical model invoking association between the solute S and α and β then it is easy to show that

$$\Delta\mu_\alpha^{\ominus,\mathrm{tr}}(T_s) = -RT_s \ln (1 + K_{\alpha s}a_s) \qquad (13)$$

where $K_{\alpha s}$ is the equilibrium constant for the process

$$\alpha + S \rightleftharpoons \alpha S \qquad (14)$$

and a_s is the activity of species S. If we assume ideality and low concentrations of S then Eqn. 13 becomes

$$\Delta\mu_\alpha^{\ominus,\mathrm{tr}}(T_s) = -RT_s K_{\alpha s} c_s \qquad (15)$$

with an analogous expression for $\Delta\mu_\beta^{\ominus,\mathrm{tr}}(T_s)$. Therefore from Eqns. 10 and 15

$$\Delta T = \left[\frac{RT_\mathrm{w}^2}{\Delta H^{\ominus,\mathrm{w}}(T_\mathrm{w})}\right] (K_{\beta s} - K_{\alpha s})c_s \qquad (16)$$

The first term in parentheses on the right-hand side of Eqns. 12 and 16 is positive and consequently whether one observes an increase or decrease in the temperature at which the phase transition occurs depends upon the difference between the interaction of the solute S with the β and α forms. A stronger interaction (i.e. more association) of the solute with the β form than with the α form will decrease the transition temperature and vice versa. Obviously if the extent of interaction of the solute S with both species participating in the phase change is the same then no perturbation in the phase transition temperature will occur. Eqn. 12 and Eqn. 16 to a greater extent have considerable limitations particularly with regard to their range of applicability. This is at present indeterminate, but one can confidently predict non-linearity in ΔT vs. c_s plots at unspecifiable but higher concentrations arising from higher order interactions of solutes with the species participating in the equilibrium and from neglect of the variation of the heat capacity with temperature associated with the transitions. Furthermore it has been assumed that the $k_{i\text{-s}}$ terms are temperature independent and this will not be quite correct. Eqns. 12 and 16 are, however, useful as limiting cases. It should be noted that if the solute S is a strong electrolyte such that 1 mol of salt gives rise to n_M mol of cation M and n_X mol of anion X Eqns. 12 and 16 become

$$\Delta T = \frac{-RT_\mathrm{w}^2}{\Delta H^{\ominus,\mathrm{w}}(T_s)}[(n_\mathrm{M}k_{\beta-\mathrm{M}} + n_\mathrm{x}k_{\beta-\mathrm{x}}) - (n_\mathrm{M}k_{\alpha-\mathrm{M}} + n_\mathrm{x}k_{\alpha-\mathrm{x}})]c_s \tag{17}$$

$$\Delta T = \frac{-RT_\mathrm{w}^2}{\Delta H^{\ominus,\mathrm{w}}(T_s)}[(n_\mathrm{M}k_{\beta\mathrm{M}} + n_\mathrm{x}K_{\beta\mathrm{x}}) - (n_\mathrm{M}K_{\alpha\mathrm{M}} + n_\mathrm{x}K_{\alpha\mathrm{x}})]c_s \tag{18}$$

where c_s is the stoichiometric salt concentration. Consequently the temperature change of a phase transition depends upon four unknown parameters for any given salt.

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