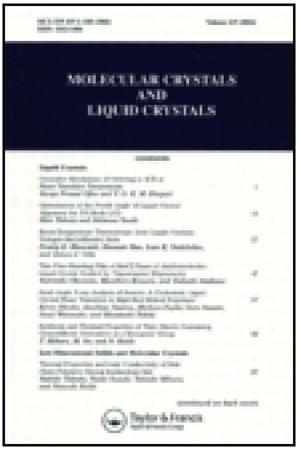

Molecular Crystals and Liquid Crystals

Effect of Monovalent Ions on the Phase Transition Behavior of DPPC-Water Dispersion

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Effect of Monovalent Ions on the Phase Transition Behavior of DPPC-Water Dispersion

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The interaction of monovalent ions and the dipalmitoyl phosphatidylcholine (DPPC) bilayer was studied using differential scanning calorimetry (DSC). Phase transition temperatures, enthalpies and entropies were determined as function of the ion-millieu. Separate attention was given to the role of cations and that of anions.

In general alkali chloride salts increase both the main transition temperature (T_m) and the pretransition temperature (T_p) of the DPPC bilayer. The T_p increasing effect correlates with the (charge)^2/radius-values of the cations. A $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$ order of effectiveness was established. In contrast, up to 3M concentration no specific order of cation effectiveness was noted on the main phase transition temperature. Each of alkali chlorides produced an increase in T_m of approximately 1.5°C at 3M concentration.

In contrast with alkali chlorides, alkali nitrates and alkali perchlorates produced no measurable effects on the DPPC phase transition temperature. These results show that chloride ions interact specifically with the DPPC bilayers and cause an increase in T_m while monovalent cations have only a secondary effect.

INTRODUCTION

Several laboratories[1-17] have studied lipid-ion interactions in the last two decades but only a few[3,6,11,13] have examined in detail the interaction of monovalent ions with uncharged lipids. However, due to the preponderance of uncharged lipids in biomembranes[18] and the presence of monovalent ions in their surroundings, the investigation

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of monovalent ion-uncharged lipid interactions is of importance from biological point of view. Previous studies on DPPC bilayers[11,13] have revealed that both monovalent cations and anions can influence the structure and thermodynamic properties of lipid bilayers. These effects may be interpreted on various bases (dehydration effects,[19] structure making and breaking effects[20] etc.) but a theoretical model to summarize the experimental data has not been developed yet.

In our work we investigated the effect of monovalent cations (Li^+, Na^+, K^+, Rb^+ and Cs^+) and anions (Cl^-, ClO_4^- and NO_3^-) on the phase transition behavior of DPPC (dipalmitoyl phosphatidylcholine)-water dispersions using differential scanning calorimetry.

MATERIALS AND METHODS

L-α-dipalmitoyl phosphatidylcholine was purchased from Sigma Chemical Co. and was used without further purification. The purity was checked by thin layer chromatography. The samples were prepared by weighing the dry lipid powder and the appropriate amount of double distilled water or salt solution in a glass vessel and then mixing at 50–60°C by vortexing for 30 minutes. Glass beads were put in the vessel to obtain homogeneous samples. The weight ratio of lipid to water was always 20%. Approx. 10 mg of the mixture was sealed hermetically in an aluminium pan.

The measurements were carried out in a DuPont 910 DSC-cell at a heating rate of 5°C/min and in the sensitivity range of 0.1–0.2 mW/cm.

The equipment was calibrated using indium. The extrapolated onset temperatures were taken as the transition temperatures. The enthalpies were calculated from the area below the endotherms measured with a planimeter.

RESULTS

At first we investigated the effect of the chloride salts of alkali cations (Li^+, Na^+, K^+, Rb^+ and Cs^+) on the phase transitions of DPPC-water dispersions.

Figure 1 shows the calorimetric scans of the DPPC suspensions at various LiCl concentrations (0–18M). Three points are evident: i) the general shape of the curves does not change; ii) there is no

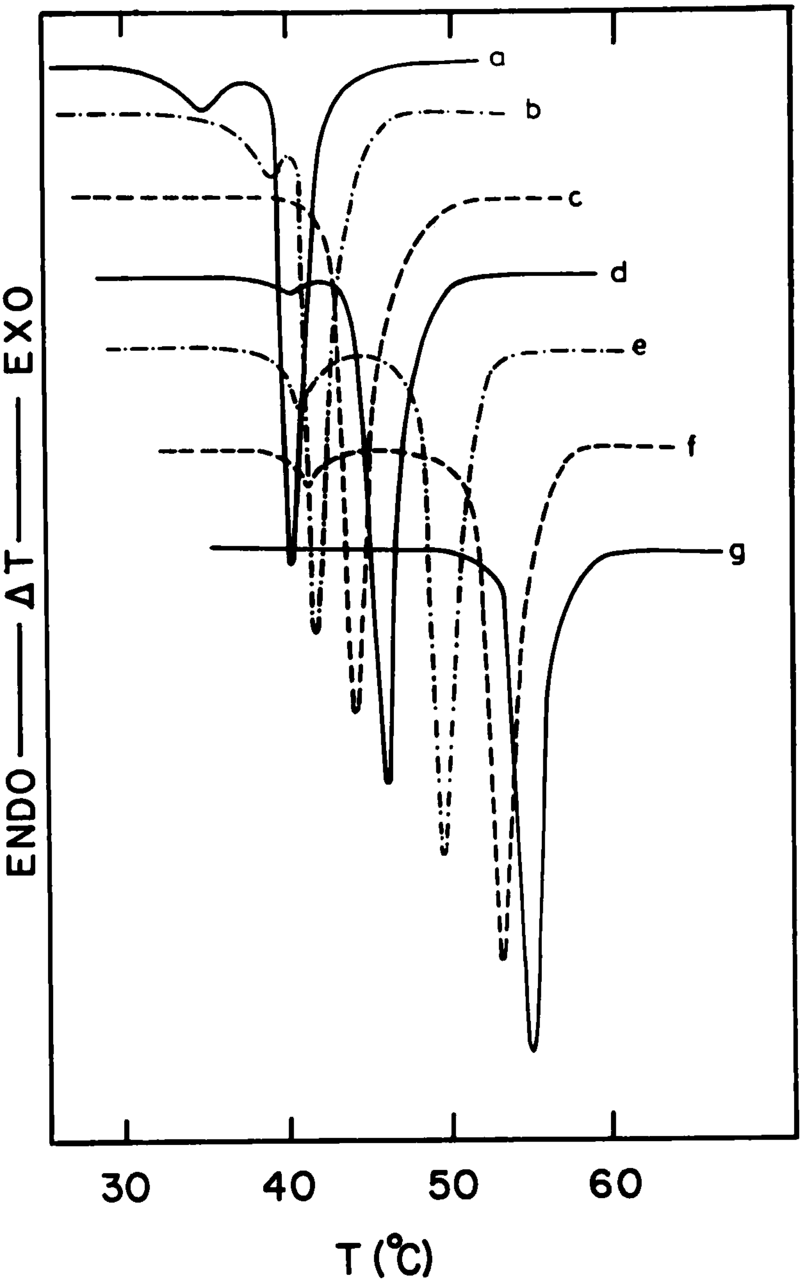

FIGURE 1 The DSC scans of DPPC-LiCl solution system. The concentration of LiCl solution are: a) 0 M b)1 M c) 3 M d) 5 M e) 9.5 M f) 11 M and g) 18 M. Heating rate 5°C/min, sensitivity 0.2 mW/cm.

broadening of either the pre-transition (T_p) or the main transition (T_m) temperature peaks; and iii) both the pre-transition and main transition temperature peaks are shifted upward with increasing LiCl concentration.

Table I illustrates in detail the effects of increasing LiCl concentrations (0–3M) on the DPPC T_p and T_m. At low salt concentrations (<0.5M LiCl), a small decrease in T_p occurs while T_m remains nearly unchanged compared to the values obtained for the pure DPPC-water systems. Other chloride salts (NaCl, KCl, RbCl and CsCl) show similar effects on the DPPC T_p and T_m at concentrations at or below 0.5M (Table I). The tendency of the change becomes more pronounced at higher concentration (0.5M to 3.0M). All the chloride salts increase both the main transition and the pretransition temperatures. The influence on T_p seems to be more pronounced than on T_m for all the chloride salts. It is interesting to note that above 1M LiCl, the pretransition temperature disappeared.

Figure 2 summarizes the concentration-dependence of T_p and T_m for the different alkali cations. Almost no differences were found between the alkali cations in their effect on T_m. However, a marked effect on T_p was noted depending on which alkali cation was used.

TABLE I

The pretransition temperatures (T_p) and Main Transition temperature (T_m) of DPPC-chloride solution systems. The errors are ± 0.2°C.

Solution	T_p (°C)	T_m (°C)	Solution	T_p (°C)	T_m (°C)
Water	35.1	41.3	0.3 M KCl	*	41.3
0.03 M LiCl	34.5	41.8	1.0 M KCl	35.4	41.7
0.1 M LiCl	33.0	41.2	1.5 M KCl	36.4	42.3
0.3 M LiCl	34.8	41.5	2.0 M KCl	37.8	42.4
0.5 M LiCl	35.5	41.5	3.0 M KCl	39.3	42.9
0.8 M LiCl	38.4	42.0			
1.0 M LiCl	39.0	42.1	0.1 M RbCl	33.5	41.3
1.5 M LiCl	—	42.2	0.3 M RbCl	33.5	41.2
2.0 M LiCl	—	42.8	1.0 M RbCl	*	41.4
2.5 M LiCl	—	42.9	1.5 M RbCl	35.5	41.6
3.0 M LiCl	—	43.1	2.0 M RbCl	36.6	42.0
			3.0 M RbCl	39.0	42.9
0.1 M NaCl	33.3	41.5			
0.5 M NaCl	34.7	41.5	0.1 M CsCl	33.5	40.7
1.0 M NaCl	36.2	41.8	0.5 M CsCl	33.0	41.0
2.0 M NaCl	37.3	42.3	1.0 M CsCl	34.0	41.2
3.0 M NaCl	39.0	43.0	3.0 M CsCl	39.5	43.0

—: the pretransition disappeared

*: it was not possible to evaluate the pretransition endotherm reliably.

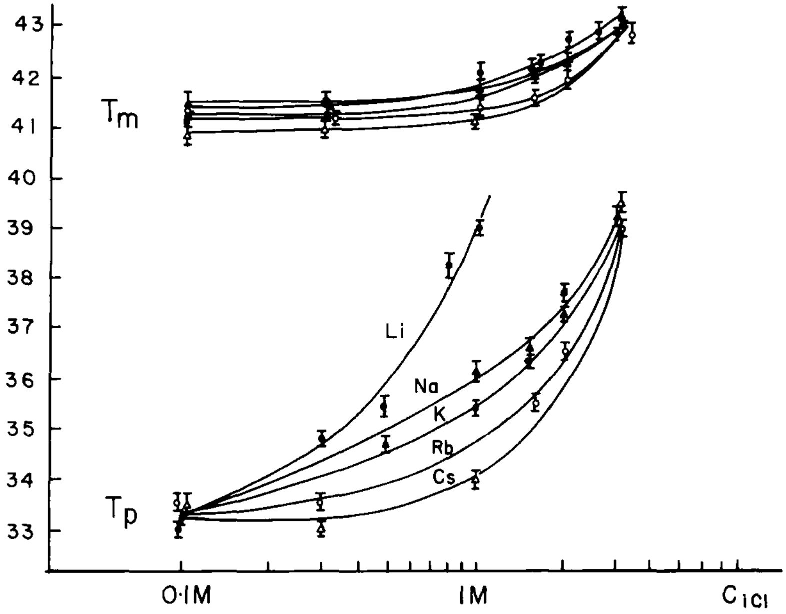

FIGURE 2 The main transition (T_m—upper curves) and pretransition temperature (T_p—lower curves) of DPPC-chloride solution systems as a function of the chloride concentration. The curve marked by -●- corresponds to LiCl, -▲- to NaCl, -■- to KCl, -○- to RbCl and -Δ- to CsCl.

The following order of effectiveness was observed in terms of shifting T_p: $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$.

Table II summarizes the enthalpy and entropy changes associated with the main transition as the concentration of various chloride salts is increased (0–3M). No changes due to the presence of salts are found if one takes into account the relatively large standard errors.

To perform a more systematic investigation which might lead to a theoretical description of DPPC interactions with the monovalent cations, we extended the salt concentration range of the new measurements. Figures 3, 4 and 5 illustrate the effect of LiCl, NaCl, KCl, RbCl, and CsCl, respectively, on the main transition temperature (T_m) of aqueous DPPC dispersions using the wider salt concentration ranges. The upper limits of the investigated ranges correspond to the solubility limits of the salts. All curves in Figures 3, 4 and 5 can be fit approximately by a linear function in the concentration range from 0M to 3M regardless of which alkali chloride is used. However, above 3M, continuation of the linear fit varies for each alkali chloride. The following order is apparent with regards to the salt concentration

TABLE II

Enthalpy (ΔH_m) and entropy changes (ΔS_m) obtained from the main transition endotherms of DPPC-chloride solution systems. The errors are indicated below.

Solution	ΔH_m (kJ.mol^{-1})	ΔS_m (J. mol^{-1} .K^{-1})
Water	41.8 ± 2.2	132.7 ± 7.0
0.1 M LiCl	42.4 ± 6.6	134.7 ± 21.0
0.3 M LiCl	44.4 ± 7.3	141.2 ± 23.4
0.8 M LiCl	38.0 ± 2.9	120.6 ± 9.3
1.5 M LiCl	34.1 ± 8.1	108.0 ± 25.6
2.0 M LiCl	39.1 ± 13.2	123.8 ± 41.8
3.0 M LiCl	41.9 ± 13.2	132.5 ± 41.8
0.1 M NaCl	40.4 ± 3.7	128.3 ± 11.7
0.5 M NaCl	42.4 ± 7.3	134.8 ± 23.3
1.0 M NaCl	40.2 ± 6.6	127.5 ± 21.0
2.0 M NaCl	42.8 ± 3.3	135.6 ± 10.5
3.0 M NaCl	37.9 ± 4.4	119.8 ± 13.9
1.0 M KCl	37.2 ± 3.3	118.2 ± 10.5
1.5 M KCl	43.8 ± 5.9	138.7 ± 18.6
2.0 M KCl	47.4 ± 5.9	150.2 ± 18.6
3.0 M KCl	41.0 ± 5.9	129.6 ± 18.6
0.1 M RbCl	42.7 ± 2.2	135.6 ± 7.0
0.3 M RbCl	38.7 ± 2.6	123.0 ± 8.2
1.0 M RbCl	37.1 ± 2.2	117.8 ± 7.0
1.5 M RbCl	42.3 ± 2.2	134.3 ± 7.0
2.0 M RbCl	39.7 ± 2.2	126.0 ± 7.0
3.0 M RbCl	39.3 ± 2.2	124.5 ± 7.0
0.1 M CsCl	41.5 ± 2.2	132.1 ± 7.0
0.5 M CsCl	42.7 ± 4.0	136.0 ± 12.9
1.0 M CsCl	40.5 ± 2.9	128.9 ± 9.3
3.0 M CsCl	40.9 ± 4.4	129.3 ± 13.9

attained before the linear fit fails: LiCl > RbCl > CsCl > NaCl > KCl. Actually, the curves for RbCl and CsCl remain nearly linear over their entire concentration ranges (Figure 5); whereas, the curves for LiCl, NaCl, and KCl go to saturating concentrations before leveling off (Figures 3 and 4).

Another interesting phenomena occurring at high salt concentrations is illustrated in Figure 1. Above 5M LiCl concentration a small but sharp endothermal peak appears beside the shifted main transition endotherm just at the temperature corresponding to the T_m of the pure DPPC-water system. It looks like a small main transition endotherm of a salt-free region of the dispersion. The peak appears at almost all concentrations above 5M, always occurs at the same temperature, and remains very small but sharp. A similar phenomenon occurs for NaCl above the concentration of 4.5M.

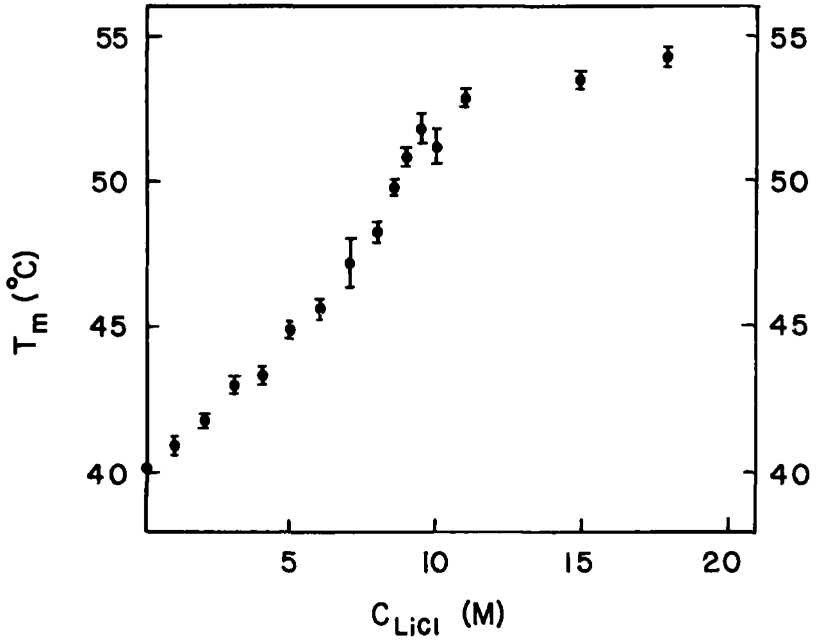

FIGURE 3 The main transition temperature (T_m) of DPPC-LiCl solution systems as a function of the LiCl concentration. It is apparent that the value for pure DPPC-water dispersion here and in Figures 4 and 5 differ from the value shown in Table I. The latter sequence of experiments was performed with lipids from different shipment of Sigma Co. The experiments with this lipid resulted in a T_m lower by ~1°C than the earlier values.

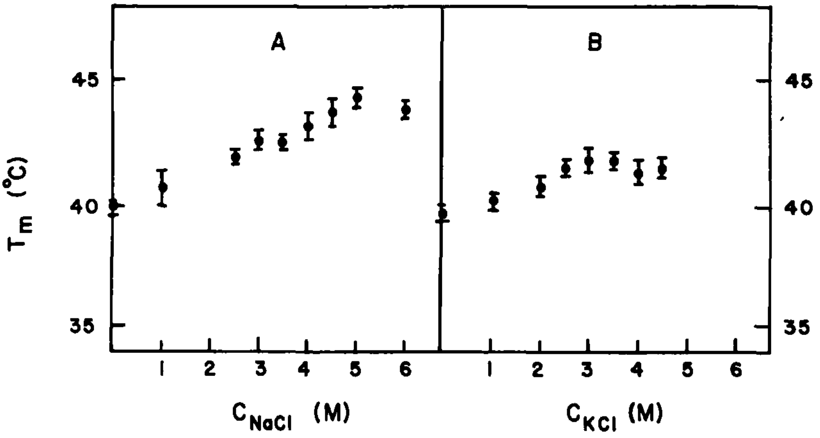

FIGURE 4 a,b The main transition temperature (T_m) of DPPC-NaCl (a) and KCl (b) solution systems as a function of the salt concentration.

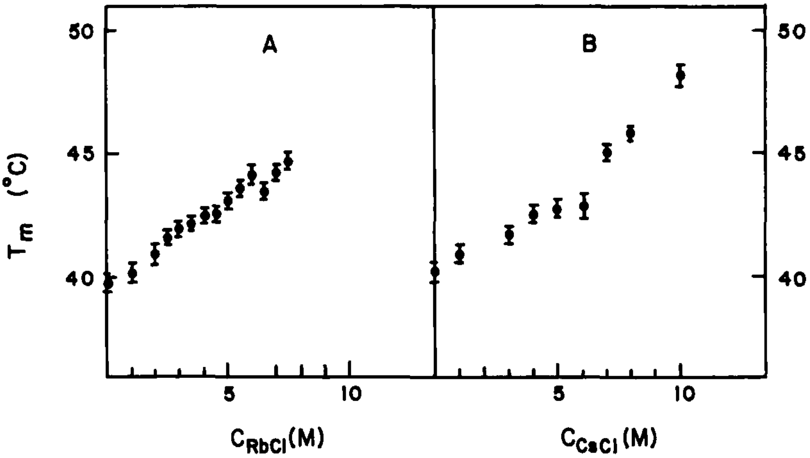

FIGURE 5 a,b The main transition temperature (T_m) of DPPC-RbCl (a) and CsCl (b) solution systems as a function of the salt concentration.

Table III shows the enthalpy and entropy changes associated with the main transition as a function of high salt concentrations. The ΔH_m and ΔS_m values do not change significantly in this wider concentration range.

Figure 6 illustrates the difficulties in the evaluation of the pretransition endotherm at higher salt concentration. The peak split into two or three smaller peaks at concentrations above 3.5M for KCl and RbCl; and 4M, for CsCl. However, the whole pretransition peak disappeared above the concentration of 1M for LiCl, and 3M for NaCl. In this respect, LiCl and NaCl are the more effective salts. The appearance of the new peaks (Figure 6), which are in the pretransition region and partly overlap the main transition endotherm, explains the uncertainty in the determination of ΔH values at higher salt concentrations (Table III). Measurements were repeated in the presence of EDTA in order to bind divalent ions possibly present. However, the same results were obtained as without EDTA molecules.

In the final part of this study, we assessed the effects of various monovalent anions (Cl^-,NO_3^-, and ClO_4^-) on the phase transition properties of DPPC-water dispersions (see Table IV). Comparing the data indicated in Table I and Table IV one can see that only the chloride salts influence the thermal parameters significantly.

TABLE III

Some ΔH_m and ΔS_m values for DPPC-chloride solution systems in wide concentration range of salts. The errors are indicated below.

Solution	ΔH_m (kJ.mol^{-1})	ΔS_m (J.mol^{-1}.K^{-1})
3 M LiCl	40.9 ± 2.9	129.4 ± 9.2
4 M LiCl	37.1 ± 3.7	117.2 ± 11.7
5 M LiCl	31.3 ± 4.4	98.4 ± 13.8
6 M LiCl	33.2 ± 4.4	104.1 ± 13.8
7 M LiCl	42.6 ± 3.7	133.0 ± 11.6
8 M LiCl	44.8 ± 5.1	139.4 ± 15.9
9 M LiCl	44.8 ± 5.5	138.3 ± 17.0
10 M LiCl	39.7 ± 3.7	122.3 ± 11.4
11 M LiCl	38.2 ± 3.7	117.2 ± 11.4
3 M NaCl	39.5 ± 4.4	125.1 ± 14.0
3.5 M NaCl	36.9 ± 3.7	116.9 ± 11.7
4 M NaCl	37.2 ± 3.7	117.6 ± 11.7
4.5 M NaCl	38.1 ± 3.7	120.2 ± 11.7
6 M NaCl	39.8 ± 5.1	125.5 ± 16.1
3 M KCl	40.1 ± 4.4	127.3 ± 14.0
3.5 M KCl	29.0 ± 3.7	92.1 ± 11.8
4 M KCl	28.0 ± 3.7	89.0 ± 11.8
4.5 M KCl	32.5 ± 3.7	103.3 ± 11.8
3 M RbCl	38.9 ± 3.7	123.5 ± 11.7
4 M RbCl	41.6 ± 4.0	131.8 ± 12.7
5 M RbCl	37.6 ± 3.7	118.9 ± 11.7
6 M RbCl	38.7 ± 3.7	121.9 ± 11.7
7 M RbCl	34.7 ± 4.8	109.3 ± 15.1
3 M CsCl	40.9 ± 4.4	129.3 ± 13.9
4 M CsCl	39.2 ± 3.0	124.1 ± 9.3
5 M CsCl	38.2 ± 2.2	120.8 ± 7.0
6 M CsCl	42.3 ± 4.8	133.8 ± 15.0
7 M CsCl	41.9 ± 5.0	131.7 ± 15.5
8 M CsCl	45.1 ± 5.0	141.2 ± 15.5
10 M CsCl	48.1 ± 5.1	149.6 ± 16.0

DISCUSSION

The interpretation of our experimental data is rather complicated because of the complexity of the interaction between lipids and ions. However, our work confirmed that both cations and anions have their role in modifying the structure and thermodynamic properties of lipid bilayers. First the effect of alkali chlorides on T_p and T_m will be discussed.

The effect of alkali chlorides on T_m is smaller than on T_p. It can

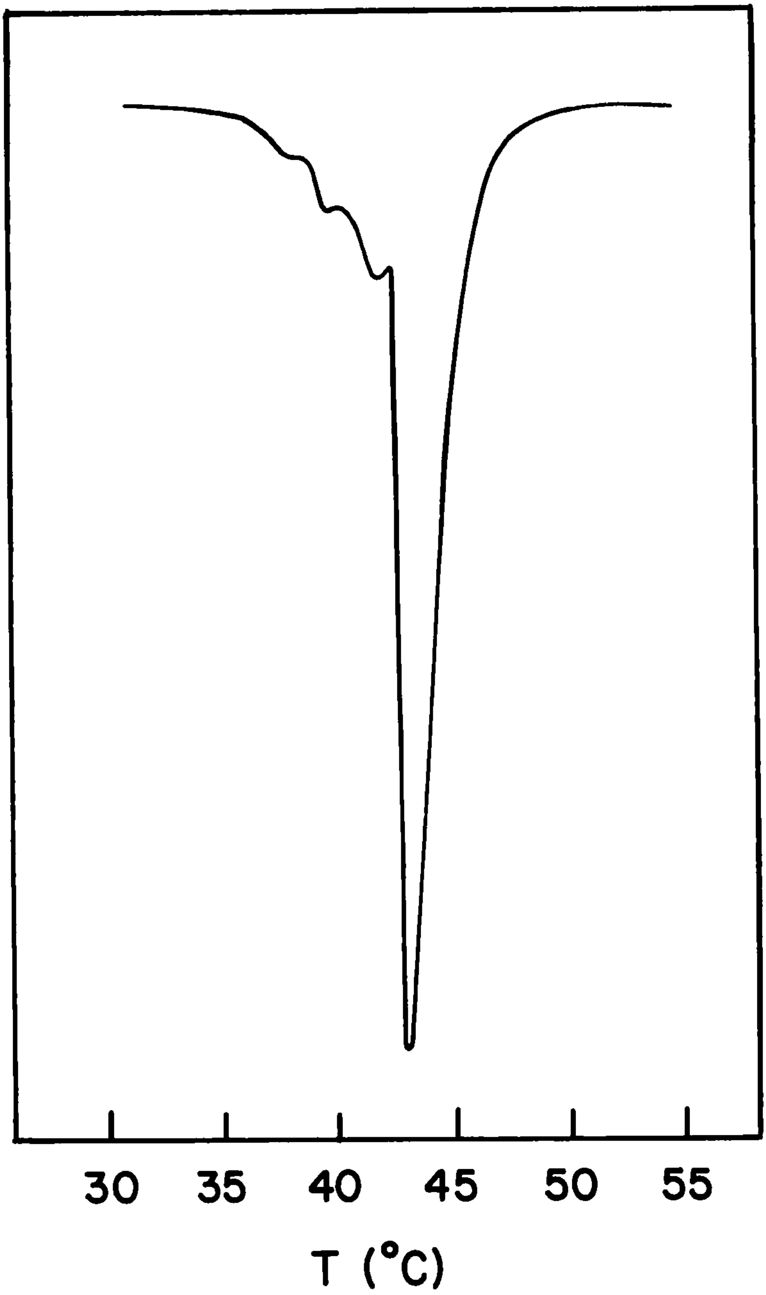

FIGURE 6 The DSC scan of DPPC-RbCl solution system at a concentration of 3.5 M of RbCl. Heating rate 5°C/min, sensitivity 0.1 mW/cm.

TABLE IV

The main transition temperature (T_m), enthalpy (ΔH_m) and entropy (ΔS_m) of DPPC-nitrate, perchlorate solution systems. The error of T_m is ± 0.2°C, the errors of ΔH_m and ΔS_m are indicated below.

Solution	Tm (°C)	ΔH_m (kJ.mol^{-1})	ΔS_m (J.mol^{-1}.K^{-1})
Water	41.0	41.8 ± 2.2	132.7 ± 7.0
1 M $LiNO_3$	40.8	42.9 ± 2.2	136.6 ± 7.0
3 M $LiNO_3$	41.3	—	—
1 M $NaNO_3$	40.5	40.4 ± 2.2	128.8 ± 7.0
3 M $NaNO_3$	41.1	—	—
1 M KNO_3	40.9	38.2 ± 2.9	121.6 ± 9.2
3 M KNO_3	41.4	45.5 ± 3.7	144.6 ± 11.8
3 M $RbNO_3$	40.9	45.5 ± 3.7	144.9 ± 11.8
1 M $CsNO_3$	40.5	43.3 ± 2.2	138.0 ± 7.0
3 M $NaClO_4$	41.4	—	—

—: we could not determine the phase transition enthalpy.

be concluded from the results illustrated in Table I and in Figure 2 that different alkali cations shift the pretransition temperature peaks varying amounts. The following sequence of alkali cations can be obtained in the 0–3M concentration range:

$$Li^+ >> Na^+ > K^+ > Rb^+ > Cs^+.$$

This sequence is in accordance with the results of Chapman et al.[11] where the authors mentioned that the association constants of alkali cations with the phosphate group follow the same order.

At the same time we could find a linear relationship between the change of the pretransition temperature (ΔT_p) and the reciprocal of the alkali cation radius ($1/r_{ion}$) (see figure 7). Since $1/r_{ion}$ is proportional to the electric polarization energy of the ion, the differences in the effect of different alkali cations can be explained by their different polarization effect on the membrane/water system. Within the same salt concentration range (<3M) the *different* effect of cations on the main transition temperature is not measurable. However, the main transition temperature of DPPC membrane depends on the type of the anion rather than the cation. While chlorides increase the main transition temperature significantly above 1M, other anions like NO_3^-, ClO_4^- do not have any effect on T_m (see Table IV). T_p values are not indicated in Table IV because the pretransition endotherms were broad and not reproducible. Consequently, a specific anion-membrane interaction is the main reason for the increasing of T_m, while cation has only a secondary effect. In the present stage of our

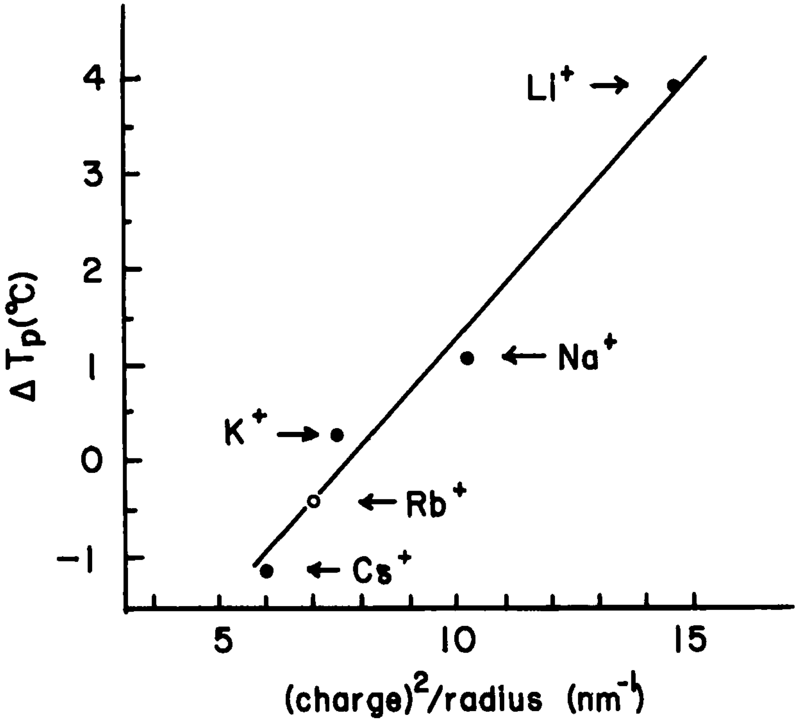

FIGURE 7 The change of the pretransition temperatures (ΔT_p) of DPPC-water system in presence of CsCl, RbCl, KCl, NaCl and LiCl at a concentration of 1 M as a function of the (charge)²/radius values of the alkali cations. The value of RbCl (o) was estimated from Figure 2. (The charge is given in a dimensionless unit.)

investigations the nature of this anion-membrane interaction is not clear. In the case of the most soluble salt (LiCl) the T_m (C_{LiCl}) curve seems to show sigmoid shape (see Fig. 3), which suggests the slightly cooperative nature of the anion-membrane interaction. Thus one can assume that the presence of the Cl^- ions results in an ordering in the membrane/water interface. The cooperative extension of this ordered H_2O, Cl^- and polar head group structure results in the increasing phase transition temperatures. Anions, which do not fit into this surface lattice structure will not promote this order.

Independently, however, of the actual nature of the interaction stabilizing the gel phase, it seems to be possible and very promising to discuss the results obtained in our experiments in the frame of the recently developed phenomenological model of Priest.[21,22]

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References

1. D. Chapman, J. Urbina and K. M. Keough, *J. Biol. Chem.*, **249**, 2512–2521 (1974).
2. H. Träuble and H. Eibl, *Proc. Natl. Acad. Sci.*, U.S.A., **71**, 214–219 (1974).
3. S. A. Simon, L. J. Lis, J. W. Kauffman and R. C. MacDonald, *Biochim. Biophys. Acta*, **375**, 317-326 (1975).
4. K. Arnold, R. Pausch, J. Frenzel and E. Winkler, *Studia Biophysics*, **51**, 81–89 (1975).
5. P. W. M. van Dijck, P. H. J. Th. Ververgaert, A. J. Verkleij, L. L. M. van Deenen and J. de Gier, *Biochim. Biophys. Acta*, **406**, 465–478 (1975).
6. Y. Inoko, T. Yamaguchi, K. Furuya and T. Mitsui, *Biochim. Biophys. Acta*, **413**, 24–32 (1975).
7. K. Jacobson and D. Papahadjopoulos, *Biochemistry*, **14**, 152–161 (1975).
8. H. Träuble, M. Teubner, P. Woolley and H. Eibl, *Biophys. Chemistry*, **4**, 319–342 (1976).
9. M. F. Brown and J. Seelig, *Nature*, **269**, 721–723 (1977).
10. J. S. Puskin, *J. Membr. Biol.*, **35**, 39–55 (1977).
11. D. Chapman, W. E. Peel, B. Kingston and T. H. Lilley, *Biochim. Biophys. Acta*, **464**, 260–275 (1977).
12. H. Hauser, C. C. Hinkley, J. Krebs, B. A. Levine, M. C. Phillips and R. J. P. Williams, *Biochim. Biophys. Acta*, **468**, 364–377 (1977).
13. E. Loshchilova and B. Karvaly, *Biochim. Biophys. Acta*, **514**, 274–285 (1978).
14. R. Kurland, C. Newton, S. Nir and D. Papahadjopoulos, *Biochim. Biophys. Acta*, **551**, 137–147 (1979).
15. M. E. Loosely-Millman, K. P. Rand and V. A. Parsegian, *Biophys. J.*, **40**, 221–232 (1982).
16. S. A. Tatulian, *Biochim. Biophys. Acta*, **736**, 189–195 (1983).
17. T. Cserháti and M. Szögyi, *Chem. Phys. Lipids.*, **34**, 93–99 (1983).
18. J. M. Boggs, *Can. J. Biochem.*, **58**, 755–770 (1980).
19. N. G. Godin and T. W. Ng, *Mol. Pharmacol.*, **9**, 802–819 (1974).
20. S. McLaughlin, A. Bruder, S. Chen and C. Moser, *Biochim. Biophys. Acta*, **394**, 304–313 (1975).
21. R. G. Priest, *Mol. Cryst. Liq. Cryst.*, **60**, 167–184 (1980).
22. I. P. Sugár and G. Monticelli, *Biophys. Chemistry*, **18**, 281–289 (1983).