S-38393, 81702-43-4; Gpp(NH)p, 34273-04-6; NaCl, 7647-14-5; dopamine, 51-61-6; *cis*(*Z*)-flupenthixol, 53772-82-0; *trans*(*E*)-flupenthixol, 53772-85-3; sodium cholate, 361-09-1.

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Modulation of the Bilayer to Hexagonal Phase Transition and Solvation of Phosphatidylethanolamines in Aqueous Salt Solutions†

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ABSTRACT: Several salts affect the temperature of the bilayer to hexagonal phase transition of phosphatidylethanolamines. Their effects are dependent on the anion as well as the cation of the salt. Salt effects on this transition can be explained by preferential hydration and ion binding. Those salts which are excluded from the solvation sphere of the membrane promote hexagonal phase formation. For example, Na_2SO_4 promotes preferential hydration and is a hexagonal phase promoter while NaSCN does not do this and is a bilayer stabilizer. Unlike amphiphiles and hydrocarbons, salts can shift the bilayer to hexagonal phase transition temperature without altering the cooperativity of the transition. The effect of these salts on the gel to liquid-crystal transition is opposite to their effect on the bilayer to hexagonal phase transition. We also find that $MnCl_2$ markedly raises the gel to liquid-crystal transition temperature. This effect is due to binding of the cation to the membrane surface. The effect is reduced with $MnSO_4$ because of preferential hydration. Our results demonstrate that the nature of the anion as well as the cation can alter the effect of salts on lipid phase transition properties. The observed effects can be explained as resulting from preferential hydration and ion binding.

Bilayers composed of phosphatidylethanolamines are particularly prone to rearrange into the hexagonal phase (Cullis & de Kruijff, 1979). The temperature at which the bilayer phase is destabilized is very sensitive to the presence of small amounts of certain hydrophobic or amphiphilic compounds (Epand, 1985a). In addition to modulation of this phase transition by substances which partition primarily into the

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membrane, the stability of the bilayer phase is also modulated by water-soluble compounds which can affect the solvation of the phospholipid head group. Sodium chloride (Seddon et al., 1983; Harlos & Eibl, 1981), chaotropic agents (Yeagle & Sen, 1986), sugar alcohols (Bryszewska & Epand, 1988), and disaccharides (Bryszewska & Epand, 1988) are among the water-soluble substances that affect the bilayer to hexagonal phase transition temperature. In the present work, we undertake a systematic investigation of the effects of a number of salts on the bilayer to hexagonal phase transition temperature of phosphatidylethanolamines. We demonstrate a relationship between the solvating properties of these salts and their effects on the phospholipid phase transitions.

Experimental Procedures

Materials. The phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL). Their purity was verified by the sharpness and temperature of their thermotropic phase transitions. All salts used were of the highest purity commercially available.

Buffer Solutions. The buffer generally used was 20 mM Pipes, 1 mM EDTA, and 150 mM NaCl with 0.002% NaN_3 at pH 7.4. To this buffer were added varying amounts of salts. However, in the case of manganese salts, hydroxide formation prevented using these salts at pH 7.4. Manganese salts were dissolved in water, and the pH was adjusted to between 4 and 2.

Phospholipid Samples for Differential Scanning Calorimetry (DSC).[1] Phospholipid was deposited as a film on the wall of a glass test tube by evaporation of the solvent from a phospholipid solution in chloroform/methanol (2/1 v/v) using a stream of nitrogen. Final traces of solvent were removed in a vacuum desiccator at 40 °C for 1 h. The lipid films were suspended in the appropriate buffer by vortexing at 45 °C for 30 s. The final lipid concentrations were 10 mg/mL for dielaidoylphosphatidylethanolamine (DEPE), 5 mg/mL for 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), and 20 mg/mL for dielaidoylphosphatidylcholine (DEPC). The lipid suspensions were degassed under vacuum before being loaded into the calorimeter.

Differential Scanning Calorimetry (DSC). Lipid suspension in buffer with salt was loaded into the sample cell, and an identical solution of buffer with salt was loaded into the reference cell of an MC-2 high-sensitivity scanning calorimeter (Microcal Co., Amherst, MA). A scan rate of 39 K/h was employed. Two heating scans were performed on each sample to ensure that the impermeant salts could equilibrate with all of the lipid by passing several times through the phase transition. Duplicate samples were also run to ensure reproducibility of the observations. In no case did we observe major alterations in thermotropic properties as a result of differences in samples or sample history. Most of our studies were done with DEPE, but similar effects of the salts were also observed with POPE. The enthalpy of the transitions of DEPE and POPE shows a variability of ±25% because these lipids do not form well-dispersed homogeneous suspensions. The salts had no substantial effect on the enthalpy of the transitions which we have previously reported (Epand, 1985b). The temperature of the main transition is reported as the temperature of maximal excess heat capacity. The bilayer to hexagonal phase transition was fitted to a single van't Hoff component and the transition temperature reported as that for the fitted curve.

[1] Abbreviations: DEPE, dielaidoylphosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; DEPC, dielaidoylphosphatidylcholine; DSC, differential scanning calorimetry; OAc, acetate; Gdn, guanidine.

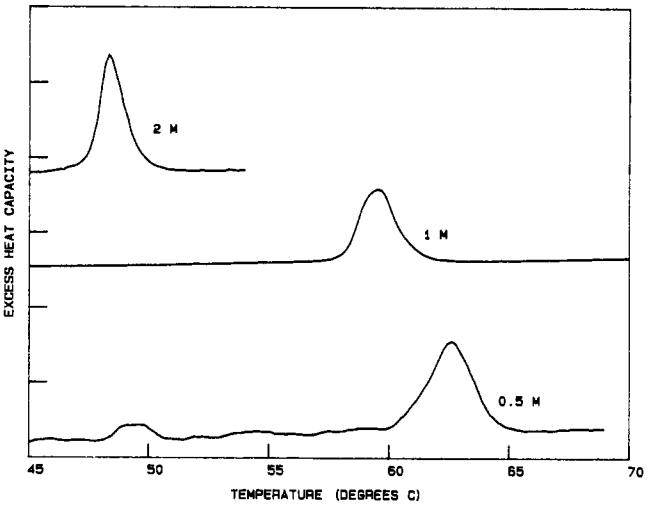

FIGURE 1: $L_\alpha \rightleftharpoons H_{II}$ DSC heating scan of DEPE (10 mg/mL) in 20 mM Pipes, 1 mM EDTA, and 150 mM NaCl with 0.002% NaN_3 at pH 7.4 and containing the indicated concentration of Na_2SO_4. Scan rate, 39 K/h. Each tick mark on the ordinate is 200 cal K^{-1} (mol of lipid)$^{-1}$.

Table I: Effect of Salts on the Phase Transition Temperatures of DEPE

salt	slope[a]	
	$L_\beta \rightarrow L_\alpha$	$L_\alpha \rightarrow H_{II}$
NaCl	1.3 ± 0.1	−5.64 ± 0.01
Na_2SO_4	1.7 ± 0.1	−9.9 ± 0.5
NaOAc	1.5 ± 0.2	−9.1 ± 0.4
NaSCN	−3.3 ± 0.5	20.0 ± 1.0
Gdn-HCl	−4.6 ± 0.5	5.0 ± 0.5
GdnHSCN	−6.5 ± 0.7	50.0 ± 2.0

[a] Slope of a plot of transition temperature vs concentration of salt. Units are degrees per salt concentration in moles per liter.

Results

The effect of several salts on the phase transition temperatures of DEPE is proportional to the concentration of salt in the lipid suspension. The effect of Na_2SO_4 on the $L_\alpha \rightarrow H_{II}$ transition of DEPE is given as an example (Figure 1). The effect of salts can be summarized by a plot of the $L_\alpha \rightarrow H_{II}$ transition temperature vs salt concentration. Positive slopes of such plots indicate which salts stabilize the lower temperature phase while negative slopes are observed for salts which lower the transition temperature (Table I). In contrast to hydrocarbons and amphiphiles which broaden the bilayer to hexagonal phase transition (Epand, 1985a), the salts studied in this work have little effect on the cooperativity of the transition, despite the fact that they can cause a marked shift in the phase transition temperature. Similar behavior is exhibited by sugars (Bryszewska & Epand, 1988). As with most additives, the effect on the gel to liquid-crystalline transition ($L_\beta \rightarrow L_\alpha$) is much less than the effect on the lamellar to hexagonal phase transition ($L_\alpha \rightarrow H_{II}$) (Table I).

The effects of some salts on the phase transitions of DEPE were not proportional to the concentration of salt. This is the case for $Gdn_2H_2SO_4$ (Figure 2). Manganese salts were also studied because of their ability to interact with solutes in water (Arakawa & Timasheff, 1984a). These salts were dissolved in water at pH 3–4. $MnSO_4$ caused a small increase in the $L_\alpha \rightarrow H_{II}$ transition temperature of DEPE, while with greater than 1 M $MnCl_2$ this transition was not observed in DEPE suspensions. The most dramatic effect of $MnCl_2$ was in raising

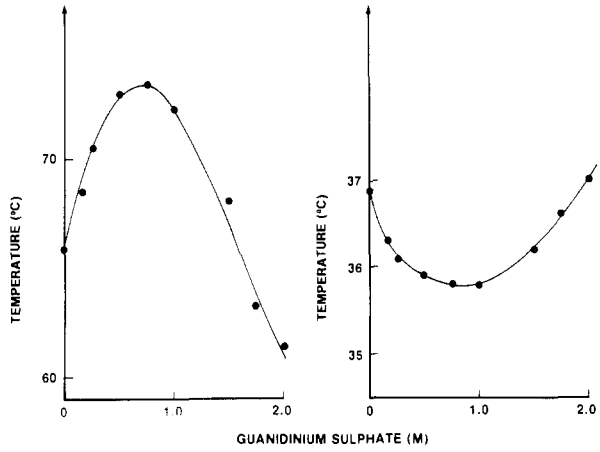

FIGURE 2: Dependence of the phase transition temperatures of DEPE on $Gdn_2H_2SO_4$ concentration. $L_\alpha \rightarrow H_{II}$ (left): $L_\beta \rightarrow L_\alpha$ (right).

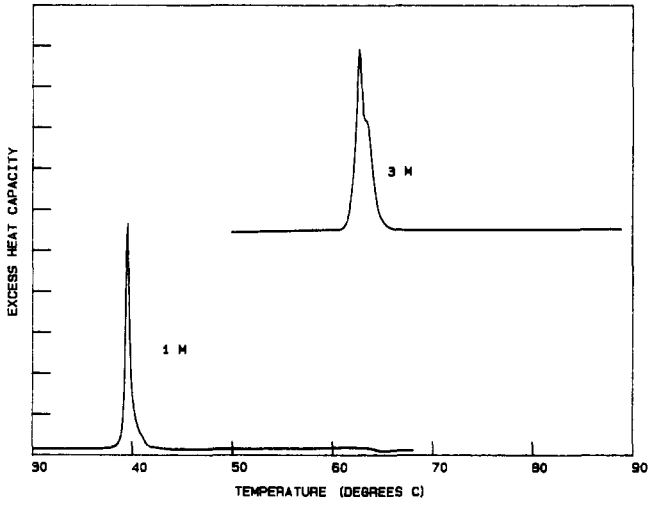

FIGURE 3: $L_\beta \rightleftharpoons L_\alpha$ DSC heating scan of DEPE (10 mg/mL) in water at pH 3–4 with either 1 or 3 M $MnCl_2$ as indicated. Scan rate, 39 K/h. Each tick mark on the ordinate is 500 cal K^{-1} (mol of lipid)$^{-1}$.

the $L_\beta \rightarrow L_\alpha$ transition temperature of DEPE (Figure 3). This effect was much weaker with $MnSO_4$ (Figure 4). Because of the marked effect of $MnCl_2$ on the $L_\beta \rightarrow L_\alpha$ transition of DEPE, its effect on the transition of other phospholipids was studied. At pH 3.0, POPE exhibits its main phase transition at 24 °C and its $L_\alpha \rightarrow H_{II}$ transition at 69 °C. In the presence of 3 M $MnCl_2$, the $L_\alpha \rightarrow H_{II}$ transition is lowered to 61.9 °C, and the $L_\beta \rightarrow L_\alpha$ transition is raised to 54 °C. This demonstrates that the effect of $MnCl_2$ is not specific to DEPE. It also illustrates an unusual phenomenon of the $L_\beta \rightarrow L_\alpha$ transition temperature being more sensitive to an additive than the $L_\alpha \rightarrow H_{II}$ transition. DEPC at pH 4 exhibits an $L_\beta \rightarrow L_\alpha$ transition at 10 °C. Addition of 1.0, 2.0, or 3.0 M $MnCl_2$ shifts the transition temperature to 14.1, 21.3, and 22.1 °C, respectively. Thus, $MnCl_2$ affects the $L_\beta \rightarrow L_\alpha$ transition of DEPC in the same direction as DEPE and POPE, but the magnitude of the effect is much smaller for DEPC.

Discussion

The effect of salts on the $L_\alpha \rightarrow H_{II}$ transition of phosphatidylethanolamines is not a result of ionic strength effects since different salts have qualitatively different effects (Table I). It is not due only to cation binding; NaCl promotes the hexagonal phase while NaSCN stabilizes the bilayer phase. It is also not a result of anion binding; the effects of NaCl and Gdn-HCl are opposite. A large part of the effect must therefore be due to changes in the solvation of the lipid in

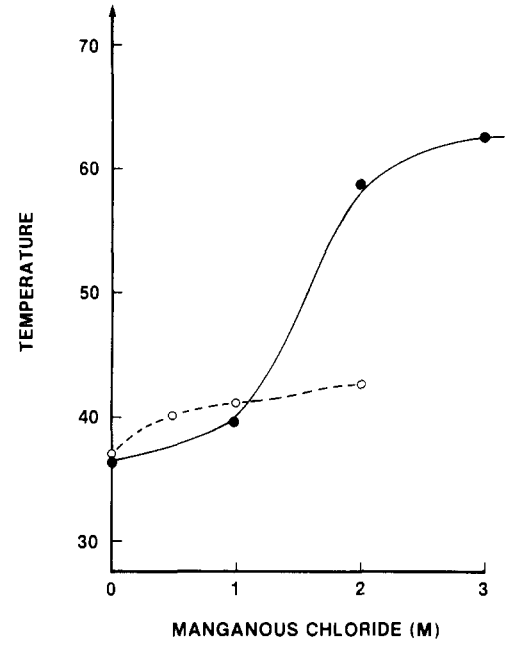

FIGURE 4: Effect of $MnCl_2$ on the $L_\beta \rightarrow L_\alpha$ transition temperature of DEPE (●). Also shown for comparison is the effect of $MnSO_4$ (○) on this transition.

solutions containing different salts. Although the systems are quite different, the effects of salts on the $L_\alpha \rightarrow H_{II}$ transition can be understood by applying concepts developed for the well-studied stabilizing and destabilizing effects of salts on protein structure. Arakawa and Timasheff (1984a) have proposed that salting-out effects are caused by a large preferential hydration of the protein. Preferential hydration describes a solute whose immediate solvation sphere is depleted of other solutes such as buffers or salts and is thus surrounded preferentially by water. The exclusion of these salts from the solvation sphere of a protein leads to an increased surface free energy at the protein–solvent interface, resulting in a more compact, folded protein conformation. This driving force to decrease the surface area of the solute will cause the lipid head groups to condense, resulting in a curvature of the lipid monolayer (Gruner, 1985). Sodium salts are among the best salting-out salts, but the effect also depends on the nature of the anion following the lyotropic series with $SCN^- < Cl^- < OAc^- < SO_4^{2-}$ in order of increasing preferential hydration. This is exactly the same as the order for increasing hexagonal phase promotion (Table I). The preferential hydration of proteins induced by sucrose (Lee & Timasheff, 1981) is also in agreement with the observed effect of sucrose in promoting hexagonal phase formation (Bryszewska & Epand, 1988). The other factor determining the effect of salts on protein stability is the binding of cations to the protein surface, thus decreasing the preferential hydration (Arakawa & Timasheff, 1984a). Gdn-HCl is a good protein denaturant because of its binding to protein surfaces (Arakawa & Timasheff, 1984b). Analogous binding of these cations to lipid head groups will increase the hydrophilicity of the membrane surface and stabilize the bilayer phase. Although it is known that ions bind to membrane surfaces (McLaughlin, 1982), the effects of salts are modulated by the preferential hydration caused by the salt. Thus, guanidinium salts are better binders to polar surfaces than sodium salts of the same anion and therefore better bilayer stabilizers (Table I). The greater preferential hydration of Gdn-HCl compared with GdnHSCN makes the latter salt a better bilayer stabilizer. The bilayer stabilizing ability of NaSCN and GdnHSCN was noted previously, but no evidence

for salt binding to the bilayer surface was obtained using NMR techniques (Yeagle & Sen, 1986). Thus, the binding of many salts is weak and transient and therefore difficult to demonstrate directly. The effect of $Gdn_2H_2SO_4$ is more complex, with this salt stabilizing the bilayer at low concentrations but promoting hexagonal phase formation at high concentrations (Figure 2). Biphasic behavior of $Gdn_2H_2SO_4$ is also observed with protein denaturation (Arakawa & Timasheff, 1984b).

Several salts of divalent cations are good salting-in agents and are also protein denaturing agents. Manganese salts are among the best of these agents (Arakawa & Timasheff, 1984a). Divalent cations also bind to membrane surfaces (Tatulian, 1987). It would therefore be expected that they would raise the bilayer to hexagonal phase transition temperature. The most dramatic effect of $MnCl_2$, however, is on the $L_\beta \rightarrow L_\alpha$ transition of phosphatidylethanolamines (Figure 4). Again, the nature of the anion is critical. Less Mn^{2+} will be bound to the membrane surface with $MnSO_4$ than with $MnCl_2$ because of the greater preferential hydration promoted by $MnSO_4$. As a result, $MnCl_2$ markedly raises the $L_\beta \rightarrow L_\alpha$ transition of DEPE and POPE, but $MnSO_4$ has little effect.

There is a very consistent finding that salts which lower the $L_\beta \rightarrow L_\alpha$ transition temperature raise the $L_\alpha \rightarrow H_\parallel$ transition temperature and visa versa (Table I). Even the biphasic effect of $Gdn_2H_2SO_4$ on the $L_\alpha \rightarrow H_\parallel$ transition is exhibited in an opposite manner on the $L_\beta \rightarrow L_\alpha$ transition (Figure 2). Also, $MnCl_2$ lowers the $L_\alpha \rightarrow H_\parallel$ transition of POPE but raises the $L_\beta \rightarrow L_\alpha$ transition of this phospholipid. The unusual feature of $MnCl_2$ is its greater effect on the $L_\beta \rightarrow L_\alpha$ transition than the $L_\alpha \rightarrow H_\parallel$ transition. The opposite directions of the shifts in transition temperatures can be understood in terms of changes in lipid head-group solvation. Interactions with the solvent which compete with lipid–lipid hydrogen bonding would increase the head-group solvation and raise the $L_\alpha \rightarrow H_\parallel$ transition temperature, but at the same time would more greatly decrease the lipid–lipid interactions in the gel than in the liquid-crystalline state and thereby lower the $L_\beta \rightarrow L_\alpha$ transition. This is opposite to the effect of dehydration which lowers the $L_\alpha \rightarrow H_\parallel$ transition temperature (Cevc, 1987) but raises the $L_\beta \rightarrow L_\alpha$ transition temperature (Seddon et al., 1983). A similar argument can be made for salts which promote preferential hydration and are excluded from the lipid surface but increase lipid–lipid interactions as a result of the increased surface tension. The result is similar to dehydration. These salts will lower the $L_\alpha \rightarrow H_\parallel$ transition temperature and raise the $L_\beta \rightarrow L_\alpha$ transition temperature.

Effects of salts on lipid phase transition properties have often been considered only as being a result of cation binding to lipid head groups. This study demonstrates that the nature of the anion as well as the cation can alter lipid phase transition properties. The observed effects appear analogous to the effects of salts on protein stability and can be interpreted as resulting from preferential hydration as well as ion binding.

Registry No. DEPE, 16777-83-6; POPE, 10015-88-0; DEPC, 52088-89-8; NaCl, 7647-14-5; Na_2SO_4, 7757-82-6; NaOAc, 127-09-3; NaSCN, 540-72-7; Gdn-HCl, 50-01-1; GdnHSCN, 593-84-0; $MnCl_2$, 7773-01-5; $MnSO_4$, 7785-87-7.

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