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The Hofmeister effect in relation to membrane lipid phase stability

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The phase behaviour of L-α-1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) was examined in aqueous dispersions containing a range of sodium salts. The phase properties of the lipid exhibited a graded response to the presence of simple anions analogous to that of the Hofmeister series encountered in the study of the solution properties of proteins. Salts early in the series (such as Na_2SO_4 and NaCl) gave rise to substantial decreases in the temperature (T_h) of the transition from the lamellar liquid-crystal to inverted hexagonal phase ($L_\alpha \rightarrow H_{II}$) and small increases in the temperature (T_m) of the transition from the lamellar gel to lamellar liquid-crystal phase ($L_\beta \rightarrow L_\alpha$). Salts towards the end of this series (such as NaI and NaSCN) led to increases in T_h and decreases in T_m. Similar effects were seen in lipid dispersions containing a series of non-ionic co-solutes. In both cases, the relative efficiency of perturbation of the lipid phase properties reflected the relative ability of the anion or co-solute to influence the structure of the bulk water. X-ray diffraction measurements indicated that these effects were probably mediated through the ability of the co-solutes to bring about changes in the extent of the lipid/water interface.

Introduction

Many biological systems are characterised by the presence of low amounts of water or by high concentrations of dissolved solutes. These include partially frozen systems of the type encountered in cryobiology, largely anhydrous systems of the type found in cryptobiology and the electrolyte-rich systems found in halobiology. There is currently considerable interest in the way in which macromolecules and macromolecular assemblies operate, or adapt to operation, under such conditions.

The properties of water as a solvent are strongly influenced by the short-range order arising from hydrogen bonding between the molecules. Its structural organisation is consequently extremely sensitive to the presence of solutes. Conversely, the structural organisation of water can influence the conformation of molecules or molecular assemblies that are dissolved or placed within it. One manifestation of this, usually referred to as the Hofmeister effect, is the graded response of proteins in solution to a series of salts of a common cation such as sodium. Different anions in such a series, may lead to the the destabilization, denaturation or solubilization of the protein whilst others may cause an aggregation and recombination of dissociated sub-units [1–4]. A typical Hofmeister series, arranged in order of increasing ability of different anions of sodium salts to solubilise (salt-in) proteins, is as follows [5]:

SO_4^{2-}; HPO_4^{2-}; F^-; Cl^-; Br^-; I^-; ClO_4^-; SCN^-.

The anions in such a series are ranked in order of increasing chaotropic activity. The ions to the right of Cl^- tend to disrupt the normal ordered structure of

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Abbreviations: POPE, L-α-1-palmitoyl-2-oleoylphosphatidylethanolamine; L_α, liquid-crystal lamellar phase; L_β, gel lamellar phase; H_{II}, inverted hexagonal phase; T_h, temperature of the $L_\alpha \rightarrow H_{II}$ transition; T_m, temperature of the $L_\beta \rightarrow L_\alpha$ transition.

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bulk water, whereas those to the left, promote alternative short-range ordered structures. The former species are normally termed chaotropes; the term kosmotrope has been coined to describe the latter species [6].

Membrane lipids constitute another important class of biological macromolecular assembly whose structures are strongly influenced by solvent interactions. This is exemplified by the phosphatidylethanolamines which readily form the inverted hexagonal phase (H_{II}), as well as the usual lamellar gel (L_β) and lamellar liquid-crystalline (L_α) phases. The phase stability of the phosphatidylethanolamines is strongly affected by a wide range of water-soluble compounds. Salts such as sodium chloride [7–9], sodium sulphate, sodium acetate [9], sugars [10,11], sugar alcohols and glycerol [10,12], all tend to lower the $L_\alpha \rightarrow H_{II}$ transition temperature (T_h) and increase the $L_\beta \rightarrow L_\alpha$ transition temperature (T_m). In contrast, potassium thiocyanate [13], sodium thiocyanate and guanidine hydrochloride [9,14], guanidine thiocyanate [9] and urea [14] all raise the value of T_h and lower that of T_m.

This study was conducted in order to determine whether the Hofmeister effect, as displayed in protein stability studies, is also manifested in membrane lipid phase stability. To do this, we studied the effects on the phase properties of L-α-1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) of hydration with a series of sodium salt solutions and compared them to the changes seen in a range of non-electrolytes of different chaotropic/kosmotropic activity. Changes in the thermodynamic parameters of the different phases were monitored using differential scanning calorimetry and structural changes were studied using X-ray diffraction techniques.

Materials and Methods

POPE was purchased from Avanti Polar Lipids (Birmingham, AL, U.S.A.) and used without futher purification. Salts and non-electrolytes were of reagent grade and solutions were prepared using deionized distilled water.

Differential scanning calorimetry (DSC). Calorimetry measurements were made using a Perkin-Elmer DSC-2 fitted with a sub-ambient accessory. Lipid samples were prepared directly in aluminium volatile-sample pans by adding solutions of the sodium salts, or non-electrolytes, to dry lipid (2:1, w/w). Full sample hydration was ensured by cycling the samples between 5°C and 40°C at 10 C° min^{-1} for an hour prior to data collection. A minimum of four heating thermograms were recorded at a scan rate of 5 C° min^{-1} for each sample. Transition temperatures were taken to be the intercept of the tangent to the rising endotherm with the baseline. After measurement, the lipid was extracted from the DSC pans using chloroform/methanol (2:1, v/v) and the amount of lipid present determined by phosphorous analysis [15].

Lipid oxidation during the course of the DSC measurements was checked for POPE dispersed in 4 M NaI. Samples cycled between 40 and 90°C at a heating/cooling rate of 10 C° min^{-1} for 3 h were analysed by TLC [12] to test for possible lipid degradation. Only traces of lysolipid were found suggesting that lipid oxidation is unlikely to be a significant problem.

X-ray diffraction. Static and real-time X-ray diffraction was conducted at station 8.2 of the Synchrotron Radiation Source at the SERC Daresbury Laboratory as described previously [16].

Lipid samples for diffraction measurements were hydrated in glass ampoules then thermally cycled using water baths to provide a similar thermal history to the calorimetric samples. The hydrated lipid was then mounted in a cell of 1 mm thickness fitted with mica windows. Temperature scans were carried out using a modified Linkam THM600 temperature-controlled microscope stage (Linkam Scientific Instruments, Surrey, U.K.).

Dynamic X-ray diffraction measurements were made at heating rates of 5 C° min^{-1}. Alternatively, the sample temperature was held constant and static X-ray diffraction patterns were recorded at fixed temperatures.

Results

In order to determine whether different anions affect the stability of lipid phases in a way analogous to their effects upon protein stability, a series of six sodium salts were used to hydrate POPE. Listed in order of increasing chaotropic ability these were Na_2SO_4, NaF, NaCl, NaBr, NaI and NaSCN.

DSC measurements

Typical thermograms of POPE hydrated in Na_2SO_4, a kosmotrope (water-structure maker), and NaI, a chaotrope (water-structure breaker), are presented in Fig. 1. They are characterised by a high enthalpy, low temperature endotherm corresponding to a lamellar gel to liquid crystal lamellar phase transition ($L_\beta \rightarrow L_\alpha$), and a low enthalpy, higher temperature endotherm corresponding to a liquid crystal lamellar to inverted hexagonal phase transition ($L_\alpha \rightarrow H_{II}$) [7]. The samples were subjected to repeated thermal cycling and thus did not exhibit the highly endothermic transitions involving crystalline sub-gel (L_c) phases sometimes observed in studies involving phosphatidylethanolamines [17,18].

The most obvious difference between the two sets of thermograms is the temperatures at which the transitions occur. Increasing concentrations of the kosmotropic salt lead to large decreases in the value of T_h

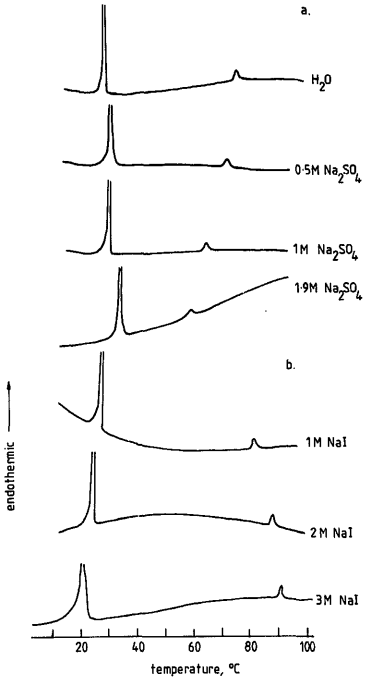

Fig. 1. Thermograms illustrating the effects of different concentrations of (a) Na_2SO_4 and (b) NaI on the phase properties of POPE. Samples, prepared as described in Materials and Methods, were run at a heating rate of 5 C° min^{-1}.

and to small increases in the value of T_m. Increasing concentrations of the chaotropic salt, in contrast, raise the value of T_h and lower the value of T_m.

The transition temperature data for POPE hydrated by the six salts used in this investigation is summarised in Fig. 2. Ordering the salts in terms of their effectiveness in lowering the value of T_h (or in the case of the chaotropes their increasing ability to raise T_h) yields the series;

Na_2SO_4 > NaF = NaCl > NaBr > NaI > NaSCN

The anions are in the same order as in the Hofmeister series for the salting-in of proteins cited in the introduction.

The results of a parallel set of experiments carried out using a range of non-ionic kosmotropes and chaotropes as co-solutes are presented in Fig. 3. They are in good agreement with earlier studies of the effects of such co-solutes on the phase properties of phosphatidylethanolamines [10–14]. Ordered as above they yield the series:

raffinose > sucrose > sorbitol > glycerol > urea > guanidine hydrochloride

This again arranges the co-solutes in terms of their decreasing kosmotropic, or increasing chaotropic properties.

The salt-induced changes in T_h and T_m are accompanied by changes in the molar enthalpies of the two transitions. Values for ΔH_m and ΔH_h, the molar enthalpy values for the $L_\beta \rightarrow L_\alpha$ and $L_\alpha \rightarrow H_{II}$ transitions, measured from thermograms of the type shown

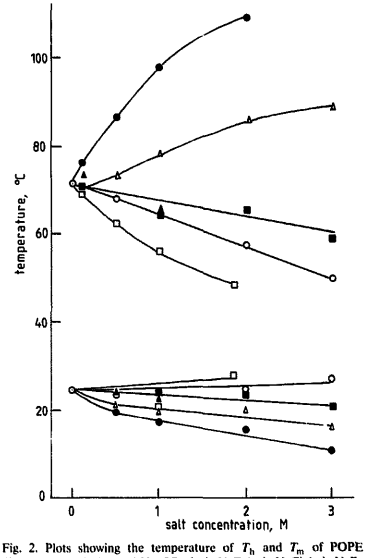

Fig. 2. Plots showing the temperature of T_h and T_m of POPE dispersed in solutions of Na_2SO_4 (□), NaF (▲), NaCl (○), NaBr (■), NaI (△) and NaSCN (●) as a function of salt concentration. Data taken from thermograms of the type shown in Fig. 1.

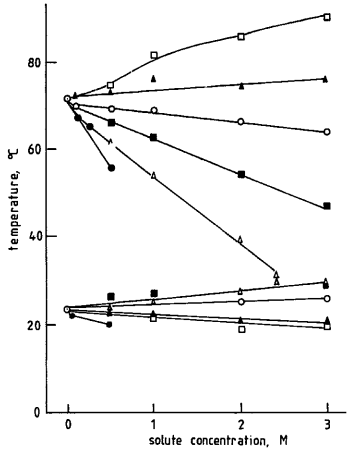

Fig. 3. Plots showing the temperature of T_h and T_m of POPE dispersed in solutions of guanidine hydrochloride (□), urea (▲), glycerol (○), sorbitol (■), sucrose (△) and raffinose (●) as a function of concentration. Data taken from thermograms of the type shown in Fig. 1.

in Fig. 1, are listed in Table I. Corresponding values of the molar entropy ΔS, calculated using the relationship $\Delta S = \Delta H/T$, where T is the temperature of the phase transition expressed in K, are also included in the table. Increasing concentrations of kosmotropic salts, as exemplified by NaCl and Na_2SO_4, leads to increasing values of ΔH_h and ΔS_h. Increasing concentrations of the chaotropic salts, as exemplified by NaSCN and NaI, show the reverse effect. No clear pattern of changes could be discerned in the values obtained for ΔH_m and ΔS_m. The detection of these latter changes is, however, severely hampered by the fact that they are of similar size to the experimental errors ($\pm 10\%$) inherent in the calorimetric measurements.

A clearer representation of the changes occurring in ΔH_h and ΔS_h is provided in Fig. 4. The trend from an increase in ΔH_h and ΔS_h values with increasing salt concentration for the early members of the Hofmeister series (NaCl, NaF and Na_2SO_4) to a decrease for the later members (NaSCN and NaI) is immediately apparent. The values for NaF solutions are for some reason all much lower than the corresponding values for the other salts. The increase in the values of ΔH_h and ΔS_h values with increasing salt concentration, characteristic of the position of NaF in the Hofmeister series, is however nevertheless clear.

X-ray diffraction

Real-time X-ray diffraction measurements were carried out under similar conditions to the DSC measurements described above. Each scan consisted of 255 individual diffraction patterns, or frames, collected continuously in real time. Three such frames of a scan of POPE hydrated with deionized distilled water corresponding to 80 °C, 50°C and 15°C are shown in Fig. 5. The first pattern indexing on the series $1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}$, corresponds to the non-bilayer H_{II} phase. The patterns measured at the two lower temperatures are characterised by maxima corresponding to the first four orders of the L_α phase and the first and fourth order of the L_β phase, respectively. The corresponding d-spacings were H_{II} (5.86 nm), L_α (5.04

TABLE I

Molar enthalpy and molar entropy values for POPE dispersed in different salt solutions

Solvent/ Salt	Concentration (M)	ΔH_m (kJ mol^{-1})	ΔH_h (kJ mol^{-1})	ΔS_m (kJ mol^{-1} K^{-1})	ΔS_h (kJ mol^{-1} K^{-1})
H_2O	–	20.5	2.01	68.8	5.82
Na_2SO_4	0.1	20.1	2.13	67.2	6.23
	0.5	18.6	2.05	62.7	6.11
	1.0	19.5	2.22	65.9	6.74
	1.9	19.8	2.55	65.7	7.95
NaF	0.1	19.9	0.46	66.7	1.34
	0.5	19.6	0.50	65.9	1.46
	1.0	14.6	0.84	49.2	2.47
NaCl	0.1	18.4	1.84	62.2	5.31
	0.5	19.5	2.26	65.9	6.61
	1.0	21.6	2.72	72.6	8.03
	2.0	18.8	2.72	63.1	8.20
	3.0	19.8	3.05	65.9	9.46
	4.0	20.4	3.56	67.5	11.2
	5.0	20.3	4.10	66.7	13.1
	6.0	19.0	4.02	62.4	12.9
NaBr	0.1	20.6	2.18	69.3	6.32
	0.5	21.6	2.01	72.8	5.86
	1.0	18.3	1.84	62.3	5.44
	2.0	19.4	1.72	65.1	5.06
	3.0	19.3	2.01	65.8	6.02
NaI	0.1	22.4	2.22	75.9	6.44
	0.5	20.3	2.13	69.0	6.15
	1.0	19.4	2.22	65.9	6.32
	2.0	19.0	1.72	64.9	4.77
	3.0	18.8	1.46	65.1	4.06
NaSCN	0.1	21.3	2.01	71.7	5.73
	0.5	21.3	1.59	72.8	4.44
	1.0	21.1	1.72	72.6	4.64
	2.0	20.6	1.09	71.3	2.85
	3.0	22.2	–	78.2	–

nm) and L_β (6.06 nm) and the wide-angle diffraction maxima were at H_{II} (0.473 nm), L_α (0.465 nm) and L_β (0.424 nm).

The transition temperatures obtained from these dynamic X-ray scans were in good agreement with those obtained from the DSC measurements. In all cases, the transitions proceeded via a two-state, or first-order, process involving the co-existence of the initial and final phases. There was no broadening in the L_α small-angle diffraction peaks prior to the $L_\alpha \rightarrow H_{II}$ transition of the type seen in systems involving intermediate phase states [19].

The effects of kosmotropic and chaotropic anions on the dimensions of the different phases were investigated by collecting a series of static X-ray patterns for POPE dispersed in different concentrations of NaCl and NaI at fixed temperatures. Patterns for the L_α, L_β and H_{II} phases were measured at 20°C, 35°C and 75°C

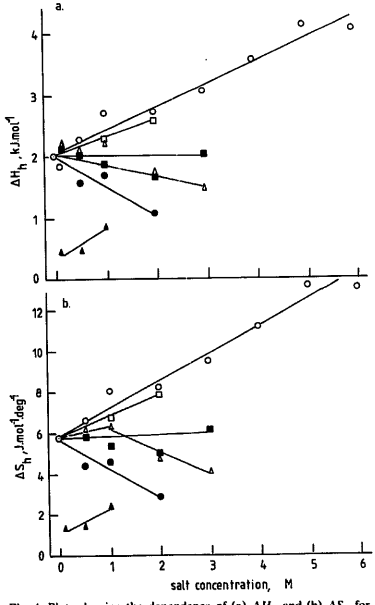

Fig. 4. Plots showing the dependence of (a) ΔH_h and (b) ΔS_h for POPE dispersed in solutions of Na_2SO_4 (□), NaF (▲), NaCl (○), NaBr (■), NaI (△) and NaSCN (●) as a function of salt concentration. Data taken from thermograms of the type shown in Fig. 1.

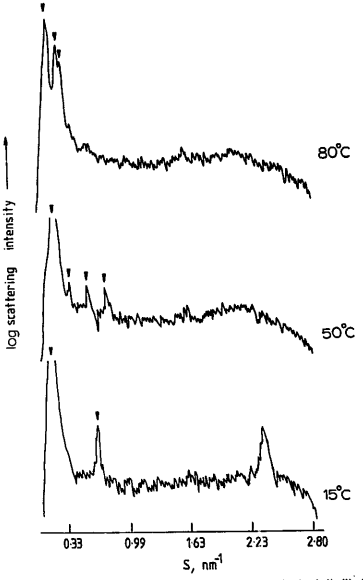

Fig. 5. Diffraction patterns of POPE dispersed in deionised distilled water at 15°C, 50°C and 80°C corresponding to the L_β, L_α and H_{II} phases, respectively. The patterns were collected during a heating scan from 10°C to 83°C carried out at a rate of 5 C° min^{-1}. The collection time for each pattern was 3.5 s.

for POPE dispersed in NaCl. Corresponding patterns for NaI dispersions were collected at 12°C, 50°C and 92°C. Plots of the *d*-spacings as a function of concentration for the two salts are presented in Fig. 6.

In both cases the *d*-spacings of the two lamellar phases increase as the salt concentration increases. This increase could, in principle, be a reflection of an increased thickness of the lipid bilayer or of the aqueous space separating the adjacent bilayers. Previous studies involving phosphatidylcholines have shown negligible change in bilayer thickness even under conditions of ion-binding [20,21]. There is, therefore, no reason to suspect that the presence of the salt is able to alter bilayer thickness. The increases in *d*-spacing of the lamellar phases are almost certainly due to a swelling of the water spaces associated with changes in the relative strengths of the attractive and repulsive forces determining bilayer separation. Control mea-

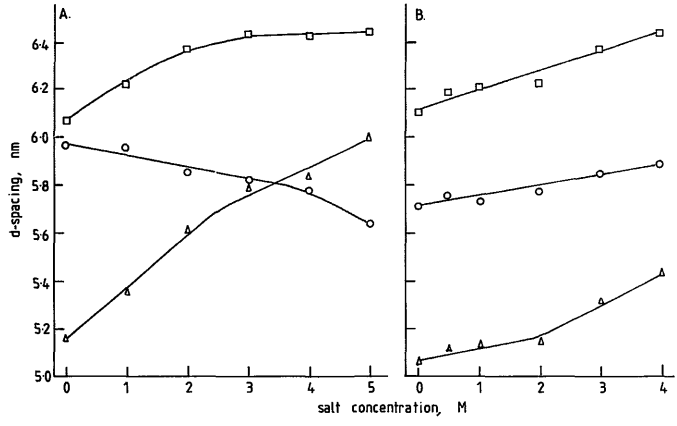

Fig. 6. Plots showing the dependence of the d-spacing of the L_β (□), L_α (△) and H_{II} (○) phases of POPE dispersed in (a) NaCl and (b) NaI on the concentration of the salts. Measurements were made at 20°C, 35°C and 75°C for lipid dispersed in the presence of NaCl, and 12°C, 50°C and 92°C for lipid dispersed in the presence of NaI, respectively.

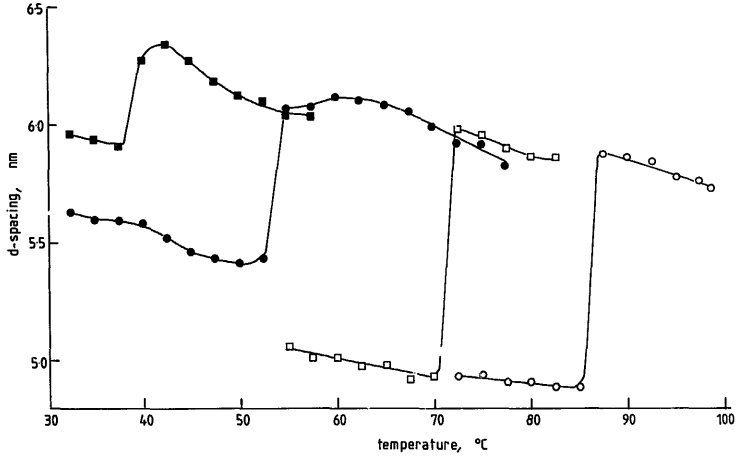

Fig. 7. Plot illustrating the temperature dependence of the d-spacing of dispersions of POPE in 6 M NaCl (■), 2 M NaCl (●), deionised water (□) and 0.5 M NaSCN (○) in the regions of their respective $L_\alpha \rightarrow H_{II}$ phase transitions.

surements, performed at higher solvent:lipid ratios (4 : 1 as opposed to 2 : 1 (w/w)), yielded identical results indicating that swelling was not limited by solvent content.

In the case of the H_{II} phase, the *d*-spacings of POPE dispersed in NaCl solutions show a decrease with increasing salt concentration while those for NaI dispersions show an increase in *d*-spacing. The possible significance of these differences is discussed below.

Plots of the temperature dependence of the *d*-spacing of the L_α and H_{II} phases formed in the presence of different concentrations of NaCl and NaSCN, measured over their respective transition ranges are shown in Fig. 7. Similar results (not shown) were obtained for the other salts. In all cases, the temperature dependence of the *d*-spacing for the fully transformed H_{II} phases of the different dispersions remained remarkably constant suggesting that no preferential adsorption of the different anions to the lipid/water interface is occurring.

Discussion

The ability of certain non-ionic co-solutes such as glycerol to stabilise proteins against thermal denaturation [22] and that of other co-solutes such as urea [23] to bring about protein denaturation are well recognised. The presence of ordered water at the protein/water interface excludes co-solutes such as glycerol from the protein surface. The resulting tendency to increase the entropy of the system is relieved by a reduction in interfacial area thus stabilising the more compact native state of the protein with respect to the more hydrated denatured state [22,24–26]. Co-solutes such as urea, in contrast, break down ordered water structures and tend to stabilise the denatured state. The precise details of these interactions, particularly when extended to ionic solutions, are still a matter of considerable debate (see Jaenicke [27] for a recent discussion). It is, however, generally recognised that kosmotropes (structure makers) favour the stability of conformations with reduced surface areas while chaotropes (structure breakers) favour those with increased surface areas.

The results reported in this paper indicate that the phase properties of membrane lipids exhibit a similar graded response to the presence of simple anions and non-ionic co-solutes to that encountered in the study of the solution properties of proteins. Salts early in the Hofmeister series (such as Na_2SO_4 and NaCl) are found to lower the temperature and raise the molar enthalpy of $L_\alpha \rightarrow H_{II}$ transitions while at the same time raising the transition temperature of the $L_\beta \rightarrow L_\alpha$. Salts towards the end of this series (such as NaI and NaSCN) have the reverse effect (Fig. 2). Similar effects are seen in lipid dispersions containing a series of non-ionic co-solutes (Fig. 3). These solutes are known to be excluded from non-solvent water associated with the lipid headgroups in liposomal systems [28] strongly suggesting that the changes seen in lipids are directly analogous to those observed for proteins.

In the case of lipids, the H_{II} phase and the L_β phases are both characterised by smaller values of area/lipid molecule than the L_α phase. While the appropriate area/lipid molecule values for POPE have not been determined, Seddon et al. [29] have, calculated surface areas per molecule for 1,2-diarachinoylphosphatidylethanolamine of 0.468 nm^2 for the $L_{\beta'}$ phase at 75°C, 0.580 nm^2 for the L_α phase at 87°C and 0.490 nm^2 for the H_{II} phase at 99°C.

The stabilisation of the H_{II} phase with respect to the L_α phase, and consequent lowering of T_h, by kosmotropes and the converse effects seen for chaotropes (Figs. 1–3), are thus consistent with the idea that the presence of kosmotropes tends to lead to a reduction in the interfacial area of the lipid while chaotropes tend to favour increases in this area. The corresponding stabilisation of L_β with respect to L_α and raising of T_m in the presence of kosmotropes and the changes in *d*-spacing of the H_{II} phase of POPE at constant temperature in the presence of kosmotropes and chaotropes, illustrated in Fig. 6, can both be similarly explained.

The increases in ΔH_h and ΔS_h for the $L_\alpha \rightarrow H_{II}$ transition, seen in the presence of kosmotropes are greater than the corresponding changes in the presence of chaotropes (Table I and Fig. 4). This again is consistent with the idea that the reduction in interfacial area, and hence in the amount of ordered water, is greater in the presence of kosmotropes than chaotropes.

Gruner and co-workers [30,31] have argued, both on theoretical and experimental grounds, that changes in the spontaneous radius of curvature of the lipid/water interface are the driving force for the formation of the H_{II} phase. They argue that the $L_\alpha \rightarrow H_{II}$ phase transition proceeds as a means to end the 'frustration' of lipid molecules residing in a bilayer without imposing excessive increases in the surface area of the lipid molecules. Thus the presence of solutes which favour reductions in lipid/water interface will tend to stabilise the H_{II} phase.

In the case of the $L_\alpha \rightarrow H_{II}$ transition, the high sensitivity of T_h to anions and non-ionic co-solutes reflects the relatively small increase in conformational disorder of the lipid chains on going from the L_α to the H_{II} phase and its partial compensation by the decrease in the amounts of ordered water associated with the headgroups of the lipids in the H_{II} phase. The much smaller changes seen in T_m presumably reflect the fact that the interfacial energy changes associated with the $L_\beta \rightarrow L_\alpha$ transition are relatively much smaller than

those associated with the increased conformational disorder of the lipid chains for this latter transition.

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