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ARTICLE

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Modulation of lipid phase behavior by kosmotropic and chaotropic solutes

Experiment and thermodynamic theory

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Abstract By means of differential scanning calorimetry and from a review of published data we demonstrate in this work that low-molecular weight kosmotropic substances (water-structure makers) of different chemical structure such as disaccharides, proline, and glycerol have identical effects on the phase behavior of several kinds of phospholipids and glycolipids. These substances favor formation of the high-temperature inverted hexagonal phase (H_{II}) and the low-temperature lamellar crystalline (L_c) and gel (L_β) phases at the expense of the intermediate lamellar liquid-crystalline phase (L_α). The latter phase may completely disappear from the phase diagram at high enough solute concentration. By contrast, chaotropic substances (water-structure breakers) such as sodium thiocyanate and guanidine hydrochloride expand the existence range of L_α at the expense of the adjacent L_β and H_{II} phases. Moreover, chaotropes are able to induce the appearance of missing intermediate liquid-crystalline phases in lipids displaying direct $L_\beta \rightarrow H_{II}$ transitions in pure water. In previous publications we have considered the influence of chaotropic and kosmotropic substances on the lipid phase behavior as a manifestation of their indirect (Hofmeister) interactions with the lipid aggregates. For a quantitative characterization of this effect, here we derive a general thermodynamic equation between lipid phase transition temperature and solute concentration, analogous to the Clapeyron-Clausius equation between transition temperature and pressure. It provides a clear description in physical quantities of the disparate effects of kosmotropic and chaotropic substances on the relative stability of the lipid-water phases. According to this equation, the magnitude of the solute effect is proportional to the hydration difference of the adjacent lipid phases and inversely proportional to the transition latent heat. The sign and magnitude of the transition shifts depend also on the degree of solute depletion (for kosmotropes) or enrichment (for chaotropes) at the interfaces, in comparison to the solute concentration in bulk water.

Key words Hofmeister effect · Clapeyron-Clausius equation · Lipid phase stability · Protectant · Denaturant · Lipid phase transition

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Introduction

As evidenced by their phase diagrams, the lipid-water systems are represented by about 20 different bilayer and non-bilayer phases. Frequently occurring among them are the lamellar crystalline (L_c) and gel (L_β) phases, which are stable at low temperatures, the lamellar liquid-crystalline phase L_α, stable at intermediate temperatures, and the inverted hexagonal phase (H_{II}), appearing at elevated temperatures. The ability of the biomembrane lipids to undergo transformations from the "fluid-bilayer" state into non-lamellar states, or into a variety of crystalline and gel phases is thought by many investigators to be involved in the mechanisms of membrane damage caused by extreme conditions (low and high temperatures, high salinity, dehydration) (Quinn 1985; Crowe et al. 1989; Bryant et al. 1992; Webb et al. 1993). On the other hand, various low-molecular substances of different chemical nature (sugars, polyols, proline) are known as natural protectants able to protect cellular membranes from injuries resulting from extreme temperatures and dehydration (Crowe and Clegg 1973; Crowe and Crowe 1982; Clegg 1983; Franks 1985; Baust 1983; Leopold 1986). In this connection, it is interesting to know the influence of these substances on the stability of the lipid phases. On the basis of a calorimetric study and a summary of previously published data, we demonstrate in the present paper that low-molecular, water-soluble protectants of different chemical structure

such as disaccharides, proline, glycerol modulate, in a qualitatively identical manner, the phase behavior of several kinds of phospholipids and glycolipids. These substances favor formation of the high-temperature H_{II} and low-temperature L_c and L_β phases at the expense of the intermediate L_α phase. At high enough protectant concentration the latter phase may completely disappear from the phase diagram. On the other side, denaturants such as sodium thiocyanate and guanidine hydrochloride, when added to the aqueous phase, expand the temperature range of the lamellar liquid-crystalline phase by moving apart the phase boundaries with the adjacent low- and high-temperature phases. Moreover, we show that denaturants can induce the appearance of missing intermediate liquid-crystalline phases in lipids displaying direct $L_\beta \rightarrow H_{II}$ transitions in pure water.

In previous studies (Koynova and Tenchov 1989; Koynova et al. 1989; Brankov and Tenchov 1993) we have related the opposite effects of protectants and denaturants to their properties as kosmotropic (water-structure makers) and chaotropic (water-structure breakers) substances, respectively. Kosmotropic and chaotropic solutes significantly influence the properties of various hydrated interfaces by the following indirect mechanism (the Hofmeister effect) (Collins and Washabaugh 1985). Kosmotropes, for example, stabilize the structure of bulk water. They tend to be excluded from interfacial regions and to reduce in this way the amount of interfacial water. In turn, this brings about a tendency to reduction of the interfacial area. In lipid-water systems, these solutes would favor the L_β and H_{II} phases at the expense of the L_α phase as the latter phases has the largest area per lipid molecule. By the same argument, chaotropes, which tend to destabilize the structure of bulk water, would support formation of the L_α phase in place of the L_β and H_{II} phases. For a quantitative substantiation of this argument, we derive in the present work a general thermodynamic relationship between lipid phase transition temperature and solute concentration, analogous to the Clapeyron-Clausius equation between transition temperature and pressure. Experimental results concerning the effects of solutes from both sides of the Hofmeister scale on the lipid phase transition parameters have been compared with the theoretical predictions.

Experimental

Materials and methods

1,2-Dihexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), all from Fluka AG (>99% pure), were used without further purification. The glycolipids 1,2-di-O-tetradecyl-3-O-β-D-glucosyl-*sn*-glycerol (14-Glc), 1,2-di-O-hexadecyl-3-O-β-D-glucosyl-*sn*-glycerol (16-Glc) and 1,2-di-O-hexadecyl-3-O-β-D-manosyl-*sn*-glycerol (16-Man) are synthetic compounds prepared as previously described (Kuttenreich 1992). The authors are indebted to Prof. H.-J. Hinz and Dr. H. Kuttenreich for the synthesis of the glycolipid compounds.

Bidistilled water or solutions of sucrose, trehalose, glycerol, proline, NaSCN, GuHCl of given concentration were added to a weighed amount of lipid. Sequential cycles of freezing-thawing were performed in combination with vigorous shaking. This procedure produces suspensions with rather good homogeneity and reproducible calorimetric behavior.

Microcalorimetric measurements were performed using high-sensitivity differential adiabatic scanning microcalorimeters DASM-1M or DASM-4 (Biopribor, Pushchino, Russia) with sensitivity better than $4 \cdot 10^{-6}$ cal $\cdot$ K^{-1} and a noise level less than $5 \cdot 10^{-7}$ W (Privalov et al. 1975). Runs were made in the temperature range 5–120 °C with heating rates of 0.5 or 1°/min. Lipid concentrations of 0.5–1 mg/ml were employed in the measurements. Usually, a given sample was scanned 3–4 times in succession. The temperature at the maximum of the excess heat capacity curve was taken as the transition temperature. The calorimetric enthalpy ΔH of the transition was determined as the area under the excess heat capacity curve. The experimental accuracy of the values for the transition temperature was estimated as $\pm 0.1-0.2$ °C and for the transition enthalpy as $\pm 5-10\%$.

Results

Phosphatidylethanolamines

Figure 1 shows representative calorimetric scans of DHPE in sucrose and NaSCN solutions of different concentrations. In excess water, the lipid displays an $L_\beta \rightarrow L_\alpha$ transition at 68.7 °C and an $L_\alpha \rightarrow H_{II}$ transition at 86.8 °C, in agreement with published data (NIST Standard Reference Database 34, 1994). An $L_c \rightarrow L_\beta$ transition at about 50 °C is also observed during the first heating after a low-temperature sample preparation or prolonged low-temperature equilibration. Sucrose reduces the temperature of $L_\alpha \rightarrow H_{II}$ transition and increases the temperature of the $L_\beta \rightarrow L_\alpha$ transition, with the former transition being more sensitive (Fig. 1A). The effects of trehalose and proline on the lipid phase transition temperatures are similar (thermograms not shown). Sodium thiocyanate, however, has the opposite effect on the DHPE transition temperatures – it reduces the temperature of the $L_\beta \rightarrow L_\alpha$ transition and increases that of the $L_\alpha \rightarrow H_{II}$ transition (Fig. 1B); a similar effect is induced by Gu.HCl (thermograms not shown). Concentrations of NaSCN above 5 M also result in splitting of the $L_\alpha \rightarrow H_{II}$ transition peak into two low-enthalpy peaks (Figs. 1B and 2). This splitting may indicate the appearance of a new intermediate phase occurring between the L_α and H_{II} phases. Taking account of the general phase sequences in aqueous dispersions of two-chain lipids and according to our preliminary X-ray data this intermediate phase appears to be a cubic liquid crystalline phase.

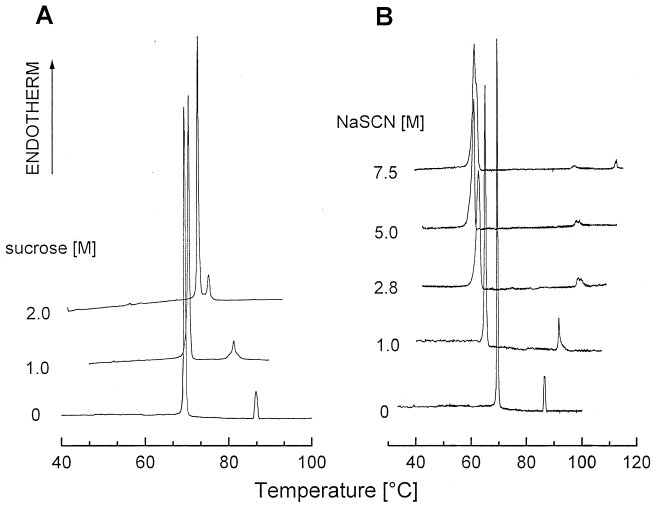

Fig. 1 Calorimetric scans of DHPE in (**A**) sucrose solutions, and (**B**) NaSCN solutions of different concentrations. The thermograms refer to second heating scans. The measurements were performed with a heating rate of 0.5 °C/min and sample concentrations of 0.5 – 1 mg/ml

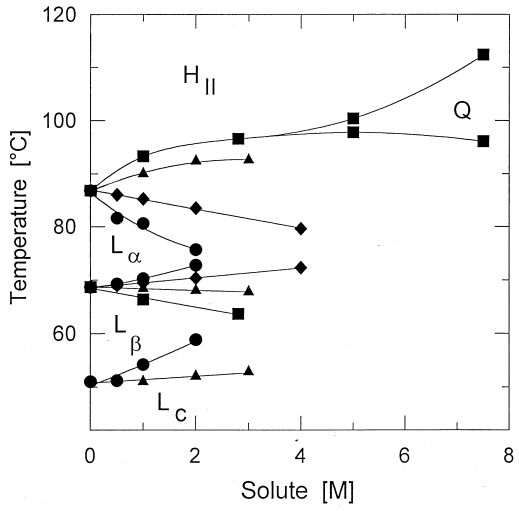

Fig. 2 Phase transition temperatures of DHPE in NaSCN (■), GuHCl (△), proline (◆) and sucrose (●) solutions. The temperature regions of existence of different phases are indicated

Table 1 summarizes the thermodynamic characteristics of the $L_\beta \rightarrow L_\alpha$ and $L_\alpha \rightarrow H_{II}$ transitions of DHPE. It is noteworthy that, besides the transition temperature, the transition enthalpy and half-width also appear to depend systematically on the sucrose or NaSCN concentration. Sucrose tends to decrease the enthalpy of the $L_\beta \rightarrow L_\alpha$ transition and to increase that of the $L_\alpha \rightarrow H_{II}$ transition, while NaSCN tends to decrease the enthalpy of the latter transition. The half-width of the transitions markedly increases upon addition of NaSCN.

Identical solute effects were also observed for two other phosphatidylethanolamine species, DPPE and DSPE (Fig. 3; Table 2). Sucrose, trehalose, and glycerol reduce the temperature of the $L_\alpha \rightarrow H_{II}$ transition and increase the temperature of the $L_\beta \rightarrow L_\alpha$ transition of DSPE. In aqueous dispersions of DPPE, an $L_\alpha \rightarrow H_{II}$ transition is not detected up to 120 °C. Addition of 1 M sucrose to the solution results in the appearance of the transition at 112 °C, while 2.4 M sucrose reduce this temperature to 82.6 °C.

Glucosyldialkylglycerols

Dialkylmonoglucosides with fatty alkyl chains 14 and 16 carbon atoms long (14-Glc and 16-Glc) and dialkylmonomannoside with alkyl chains 16 carbon atoms long (16-Man) were studied. An aqueous dispersion of 14-Glc exhibits an $L_\beta \rightarrow L_\alpha \rightarrow H_{II}$ phase sequence ($L_c \rightarrow L_\alpha \rightarrow H_{II}$ during the first heating after low-temperature sample preparation or after prolonged storage at low temperature), with transition temperatures as given in Table 3, well in accord with previous studies (Hinz et al. 1991; Tenchova et al. 1996). Low concentrations of trehalose or sucrose in the aqueous medium result in a narrowing of the temperature range for existence of the L_α phase (Fig. 4). At concentrations of 0.33 M trehalose or 1 M sucrose, the L_α phase is fully suppressed and a direct $L_\beta \rightarrow H_{II}$ transition takes place. Further increase of the sugar concentration results in increasing the temperature of the $L_\beta \rightarrow H_{II}$ transition. As with phosphatidylethanolamines, NaSCN has the opposite effect on the phase transition temperatures of 14-Glc (Fig. 4A). Dispersions of 16-Glc and 16-Man in

Table 1 Thermodynamic parameters of the phase transitions of DHPE at different concentrations of NaSCN and sucrose

Solute concentration	Lamellar gel – lamellar liquid crystal			Lamellar liquid crystal – nonlamellar		
[M]	T_m [°C]	ΔH [kcal/mol]	$\Delta T_{1/2}$ [°C]	T_h [°C]	ΔH [kcal/mol]	$\Delta T_{1/2}$ [°C]
NaSCN						
0	68.7 ± 0.0	7.83 ± 0.32	0.3	86.8 ± 0.1	1.49 ± 0.12	0.7
1	66.4 ± 0.1	7.59 ± 0.38	0.6	91.6 ± 0.2	1.88 ± 0.46	0.5
2.5	62.7 ± 0.0	7.29 ± 0.47	1.2	98.7 ± 0.2	1.09 ± 0.12	2.1
5	61.0 ± 0.1	7.78 ± 0.34	1.1	97.9 ± 0.4 / 99.1 ± 0.3	0.65 ± 0.17	1.1
7.5	60.4 ± 0.1	7.38 ± 0.52	1.9	97.5 ± 0.3 / 112.5 ± 0.5	0.27 ± 0.12 / 0.42 ± 0.23	1.5 / 0.8
Sucrose						
0	68.7 ± 0.0	7.83 ± 0.32	0.3	86.8 ± 0.1	1.49 ± 0.12	0.7
0.5	69.3 ± 0.1	7.52 ± 0.23	0.3	81.6 ± 0.3	1.56 ± 0.21	0.6
1	70.4 ± 0.1	7.03 ± 0.37	0.4	76.3 ± 0.3	1.77 ± 0.15	0.7
2	72.5 ± 0.1	5.91 ± 0.62	0.4	75.1 ± 0.4	1.96 ± 0.18	0.6

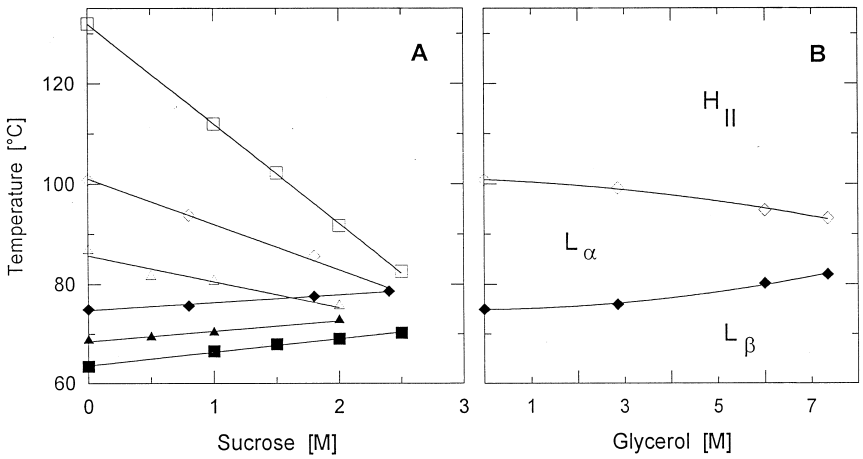

Fig. 3 Phase transition temperatures of (**A**) DHPE (▲, △), DPPE (■, □), and DSPE (◆, ◇) in sucrose solutions; (**B**) DSPE in glycerol solutions

Table 2 Transition temperatures of phosphatidylethanolamines at different solute concentrations

Lipid	Solute [M]		Transition temperatures [°C]			
			L_c–L_β	L_c–L_α	L_β–L_α	L_α–H_{II}
DHPE	–		51.0		68.7	86.8
	sucrose	0.5	51.2		69.3	81.6
		1	54.2		70.4	76.3
		2	58.9		72.5	75.1
	trehalose	0.65			69.7	79.7
		1.4			70.3	74.8
		1.8			71.3	
	proline	0.5	51.9		69.0	86.0
		1	53.2		69.5	85.2
		2	55.8		70.4	83.5
		4	59.0		72.3	79.6
	NaSCN	1	51.0		66.4	91.6
		2.5	50.8		62.7	98.7
		5	49.3		61.0	97.9, 99.1
		7.5	44.7		60.4	97.5, 112.5
	GuHCl	1	51.0		68.4	90.0
		2	52.0		68.1	92.4
		3	52.8		67.8	92.6
DPPE	–				63.4	
	sucrose	1			66.1	112.0
		1.5			67.9	102.2
		2			69.0	91.7
		2.5			70.2	82.6
DSPE	–			74.2	75.0	100.8
	sucrose	0.8		75.3	75.7	93.8
		1.8		78.4	77.6	85.6
		2.4				78.6[a]
	trehalose	0.8		75.4	76.0	94.0
		1.4		76.6	76.6	88.6
	glycerol	1		74.3	75.3	100.2
		2.85		74.5	76.0	99.2
		6		74.8	80.2	94.8
		7.35		75.6	82.0	93.2

[a] Direct L_β–H_{II} transition

water exhibit a direct $L_\beta \rightarrow H_{II}$ transition (Hinz et al. 1991; Tenchova et al. 1996). During the first scan after a low-temperature sample preparation or after a low-temperature equilibration 16-Glc also exhibits an $L_c \rightarrow L_\beta$ transition at ~54 °C. Addition of NaSCN to the aqueous phase induces the appearance of an additional low-enthalpy transition at temperatures above the main chain-melting transition in both glycolipids (Figs. 5 and 6). Our preliminary X-ray diffraction measurements show that the intermediate phase between the two transitions is a mixture of L_α and cubic phases, with dominating L_α and only traces of cubic upon heating, and dominating cubic on cooling.

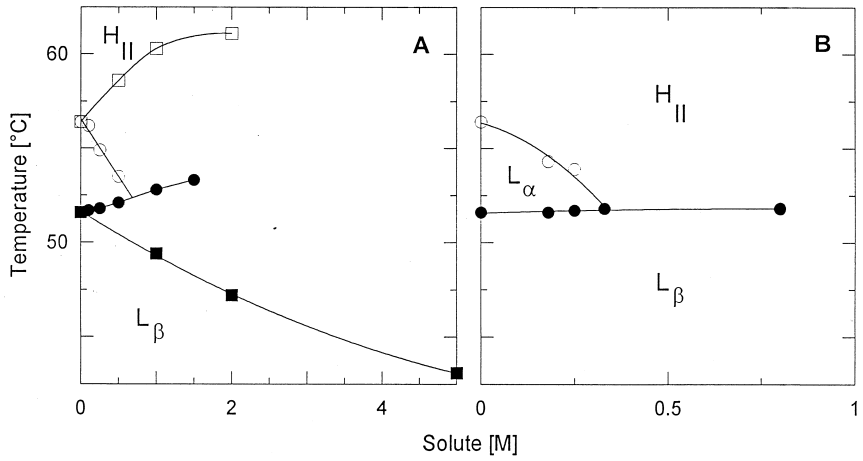

Fig. 4 Phase transition temperatures of 14-Glc in (**A**) sucrose (●, ○) and NaSCN (■, □) solution; (**B**) trehalose solutions (●, ○)

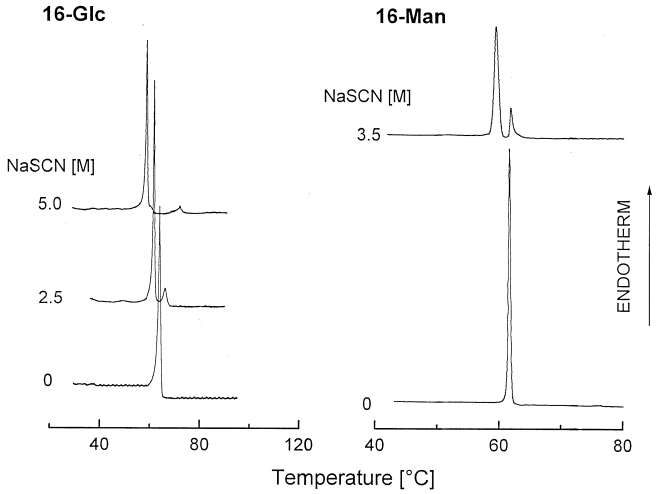

Fig. 5 Calorimetric scans of 16-Glc (*left panel*) and 16-Man (*right panel*) in water and NaSCN solutions. The thermograms refer to second heating scans. The measurements were performed with a heating rate of 0.5 °C/min and sample concentrations of 0.5–1 mg/ml

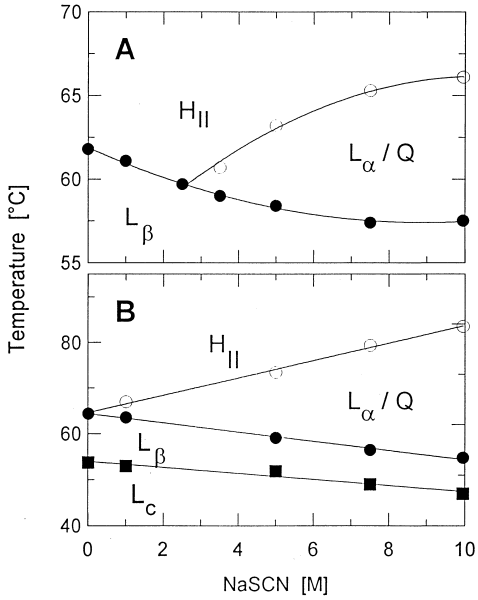

Fig. 6 Phase transition temperatures of 16-Man (**A**) and 16-Glc (**B**) in NaSCN solutions. The temperature regions of existence of different phases are indicated

Upon increasing the concentration of NaSCN, the chain-melting transition decreases in temperature while the high-temperature one increases. The transition temperature values are summarized in Table 3. The trends of the transition temperatures as functions of the solute concentration are shown on Fig. 6.

Dipalmitosylphosphatidylcholine

Addition of sucrose or proline to the aqueous dispersions of DPPC results in shifts of the pretransition ($L_{\beta'} \rightarrow P_{\beta'}$) and the main transition ($P_{\beta'} \rightarrow L_\alpha$) to higher temperatures. The former transition is more sensitive to solutes than the latter one. As a consequence, the pretransition merges with the main transition at high solute concentration. Sucrose and proline at concentrations of 2.4 M and 3 M, respectively, fully suppress the rippled $P_{\beta'}$ phase, as demonstrated in Fig. 7. The transition temperatures of DPPC at different concentrations of sucrose and proline are summarized in Table 4.

Thermodynamic theory

In order to describe the observed solute effects on the transition temperatures, here we derive a general thermodynamic relationship of the Clapeyron-Clausius type that relates the change in the transition temperature T_{tr} to the change in the concentration c of a solute C in the aqueous solution, i.e. we express the ratio dT_{tr}/dc as a function of the thermodynamic characteristics of the lipid-water system.

Table 3 Transition temperatures of glycoglycerolipids at different solute concentration

Lipid	Solute [M]		Transition temperatures [°C]				
			L_c-L_β	L_c-L_α	$L_\beta-L_\alpha$	$L_\beta-H_{II}$	$L_\alpha-H_{II}$
14-Glc	–			51.7	51.6		56.4
	trehalose	0.18			51.6		54.3
		0.25			51.7		53.9
		0.33				51.8	
		0.8				51.8	
	sucrose	0.1		51.8	51.7		56.2
		0.25		52.0	51.8		54.9
		0.5		52.3	52.1		53.5
		1		53.0		52.8	
		1.5		53.5		53.3	
	NaSCN	0.5		51.2	50.5		58.6
		1		49.7	49.4		60.3
		2		48.8	47.2		61.1
		5		44.9	43.1		–
16-Glc	–		53.8			64.4	
	NaSCN	1	53.0		63.6		67.0
		5	51.9		59.2		73.5
		7.5	49.0		56.5		79.4
		10	47.0		54.8		83.5
16-Man	–					61.8	
	NaSCN	1				61.1	
		2.5			59.7		59.7
		3.5			59.0		60.7
		5			58.4		63.2
		7.5			57.4		65.3
		10			57.5		66.1

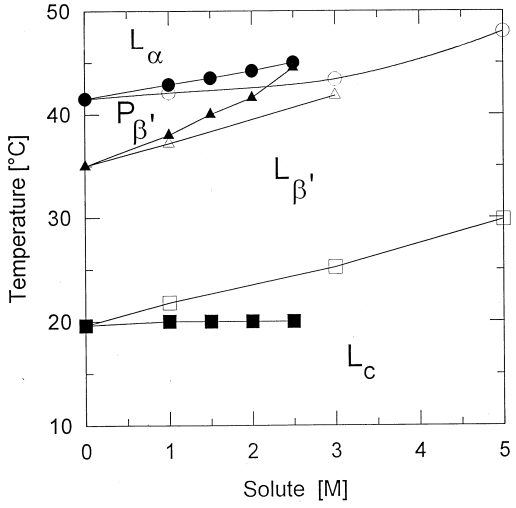

Fig. 7 Phase transition temperatures of DPPC in sucrose (*full symbols*) and proline (*open symbols*) solutions. The temperature regions of existence of different phases are indicated

Table 4 Transition temperatures of DPPC at different solute concentrations

Lipid	Solute [M]		Transition temperatures [°C]		
			$L_c-L_{\beta'}$	$L_{\beta'}-P_{\beta'}$	$P_{\beta'}-L_\alpha$
DPPC					
	–		19.6	35.0	41.5
	Sucrose				
		1	20.0	38.0	42.9
		1.5	20.0	40.0	43.5
		2	20.0	41.6	44.2
		2.4	20.0	44.5	45.0
	Proline				
		1	21.8	37.2	42.1
		3	25.2	41.8	43.4
		5	29.8	48.0[a]	

[a] Direct $L_{\beta'}-L_\alpha$ transition

The lipid-water systems investigated in the previous section are two-phase, three-component systems. The lipid phase coexists with a phase of bulk water which is in large excess. The three components are lipid, water, and solute molecules. At the phase transition temperatures, the lipid-water systems become three-phase systems with two coexisting lipid phases in equilibrium with the excess water phase. We consider a model thermodynamic system S that consists of an aggregate of N_L lipid molecules together with N_w molecules of associated water (interfacial or interlamellar water) and N_c molecules of solute species C. This system is kept at mechanical and thermal equilibrium at pressure p and temperature T. It is assumed to be in material contact with a reservoir R of excess water solution of species C molecules. In order to simplify the arguments, we neglect the lipid solubility in the excess water phase as it is extremely low compared to that in the lipid phase. It is natural to work in a semigrand ensemble in which the thermodynamic state of the system S is completely de-

scribed by one extensive quantity, the number of lipid molecules N_L, and four intensive variables: pressure p, temperature T, and chemical potentials μ_w and μ_c, of the water and solute molecules, respectively. About the various applications of semigrand ensembles one may consult the paper by Alberty and Oppenheim (1989). Another approach to this problem may be based on accounting for various microscopic interactions of the solute and water molecules with the lipid as mutual perturbation in the chemical potentials of these species [Cevc and Marsh 1987; Chapter 8, and references therein]. However, owing to the complexity of these interactions, such an approach inevitably includes a large number of explicit and implicit approximations. The alternative, purely thermodynamic approach suggested here considers the manifestation of the underlying microscopic interactions in terms of macroscopic, measurable thermodynamic quantities such as changed water and solute concentrations in the lipid phase.

Starting from the Gibbs free energy $G(p, T, N_1, N_2, N_3)$ for a 3-component system consisting of fixed numbers N_i of molecules of each species, $i=1, 2, 3$, one can define a semigrand free energy $G^{(1)}$ describing a system closed with respect to the lipids, $N_1=N_L$, and open with respect to the remaining species, $N_2=N_w$ and $N_3=N_c$. By means of the Legendre transformation we obtain

$$G^{(1)}(p, T, N_L, \mu_w, \mu_c) = N_L \mu_L(p, T, N_L, \overline{N_w}, \overline{N_c}) = N_L \mu_L(p, T, \mu_w, \mu_c) \quad (1)$$

where $\overline{N_{w,c}} = \overline{N_{w,c}}(p, T, N_L, \mu_w, \mu_c)$. The total differential of $G^{(1)}$ is given by

$$dG^{(1)} = -S\,dT + V\,dp + \mu_L\,dN_L - \overline{N_w}\,d\mu_w - \overline{N_c}\,d\mu_c \quad (2)$$

All the thermodynamic quantities of interest can be obtained by taking the appropriate derivatives of $G^{(1)}$, see Eq. (2).

Consider now the region of a first-order phase transition, where two phases P′ and P″ of the system S coexist. We distinguish the quantities pertaining to phase P′ (P″) by furnishing the corresponding symbols with a prime (second) sign. Since the phase transition takes place at constant pressure p, temperature T_{tr}, number of lipid molecules N_L, and chemical potentials μ_w, μ_c of the reservoir, the phase equilibrium condition becomes

$$\mu_L'(p, T_{tr}, \mu_w, \mu_c) = \mu_L''(p, T_{tr}, \mu_w, \mu_c) \quad (3)$$

This condition defines the transition temperature T_{tr} as an implicit function of p, μ_w and μ_c. Differentiation of Eq. (3) with the use of Eq. (2) leads to the relationship

$$V'\,dp - S'\,dT - \overline{N_w'}\,d\mu_w - \overline{N_c'}\,d\mu_c = V''\,dp - S''\,dT - \overline{N_w''}\,d\mu_w - \overline{N_c''}\,d\mu_c \quad (4)$$

Now we need to specify the dependence of the chemical potentials μ_w and μ_c on the solute concentration c. Let N_w^T and N_c^T be the total numbers of water and solute molecules in the closed system composed of the lipid aggregate S and the reservoir R and let $\overline{N_w^R} = N_w^T - \overline{N_w}$ and $\overline{N_c^R} = N_c^T - \overline{N_c}$ be the average numbers of water, $\overline{N_w^R}$, and solute, $\overline{N_c^R}$, molecules in the reservoir. We assume low molar concentration c of species C in the reservoir,

$$c = \overline{N_c^R}/(\overline{N_w^R} + \overline{N_c^R}) \approx N_c^T/N_w^T \ll 1 \quad (5)$$

and adopt the law for ideal solutions to model the chemical potentials μ_w and μ_c. Hence, for the total differentials $d\mu_w$ and $d\mu_c$ we obtain:

$$d\mu_w = v_w(p, T)\,dp - [s_w(p, T) + k_B c]\cdot dT - k_B T\cdot dc \quad (6)$$
$$d\mu_c = v_c(p, T)\,dp - [s_c(p, T) - k_B \ln c]\cdot dT + k_B T\cdot dc/c$$

Here $v_w(p, T)$ and $v_c(p, T)$ are the specific volumes of water and species C in the reservoir, $s_w(p, T)$ and $s_c(p, T)$ are the specific entropies of the corresponding pure substances, $k_B c$ and $-k_B \ln c$ are the contributions to the specific entropies due to mixing.

Table 5 Effect of the studied solutes on the temperatures of the different lipid phase transitions

Solute	Lipid	Slope (dT_{tr}/dc) [K/M]					
		L_c-L_β	L_c-L_α	$L_{\beta'}-P_{\beta'}$	$L_\beta-L_\alpha$	$L_\beta-H_{II}$	$L_\alpha-H_{II}$
Sucrose	DPPC	0.4		3.0	1.4		
	DHPE	0.4			1.2		−10.4
	DPPE				2.7		−19.6
	DSPE		1.4		0.9		−8.8
	14-Glc		1.0		1.2		−3.8
Trehalose	DHPE				1.5		−11.0
	DSPE		1.5		1.3		−8.5
	14-Glc				0.3		−10.7
Proline	DPPC	2.0		2.2	0.6		
	DHPE	1.8			0.6		−1.6
Glycerol	DSPE		0.1		0.4		−0.6
NaSCN	DHPE	0.0			−2.3		4.8
	14-Glc		−1.0		−2.2		4.4
	16-Glc	−0.8			−1.1		1.6
	16-Man				−0.7	−0.7	1.0
GuHCl	DHPE	0.0			−0.3		3.2

Finally, taking into account the conservation of the total number of particles, we obtain the following extended version of the Clapeyron-Clausius equation:

$$\Delta V^T dp - \Delta S^T dT + k_B T \left[\Delta N_w^S - \frac{1}{c} \Delta N_c^S \right] dc = 0 \qquad (7)$$

Here ΔV^T and ΔS^T are the total changes in the volume and entropy, respectively, ΔN_w^S and ΔN_c^S denote changes in the numbers of water and solute molecules in the system S, respectively.

If the phase transition takes place at constant pressure, a general thermodynamic equation for the change of the transition temperature $T = T_{tr}$ with the change in the concentration c follows from Eq. (7) at $dp = 0$:

$$\frac{dT_{tr}}{dc} = \frac{k_B T_{tr}^2}{Q_{tr}} \left[\Delta N_w^S - \frac{1}{c} \Delta N_c^S \right] \qquad (8)$$

where $Q_{tr} = T_{tr} \Delta S^T$ is the total latent heat of the transition. From this equation it is seen that the change of the transition temperature has been expressed as a linear combination of the differences between the average numbers of water and solute molecules associated with each of the lipid phases. These differences arise from the presumably different preferential interactions of the above species with the distinct lipid phases. If one relaxes the condition for small solute concentrations c and replaces the assumption for an ideal solution in Eq. (6) by a more general one, for example that for a regular solution, then the right-hand side of Eq. (8) is modified by an additive correction term that depends on the nonideality parameter λ:

$$\left(\frac{dT_{tr}}{dc} \right)_{\text{reg}} = \left(\frac{dT_{tr}}{dc} \right)_{\text{ideal}} + \frac{k_B T_{tr}^2}{Q_{tr}} \cdot \left\{ c \left[\frac{1}{1-c} - \frac{2\lambda}{k_B T_{tr}} \right] \Delta N_w^S + \frac{2\lambda}{k_B T_{tr}} (1-c) \Delta N_c^S \right\} \qquad (9)$$

As expected, this term becomes negligible at low solute concentrations where Eq. (9) transforms into Eq. (8). Let us continue the analysis of Eq. (8). By introducing for each phase the corresponding fraction of interfacial water per lipid molecule, $x = N_w/N_L$, and solute concentration in the interfacial water, $c = N_c/N_w$, we obtain

$$\frac{dT_{tr}}{dc} = \frac{RT_{tr}^2}{\Delta H} \left[(1 - c'/c) x' - (1 - c''/c) x'' \right] \qquad (10)$$

where ΔH is the transition latent heat per mol lipid and R is the gas constant. The prime and second signs denote the high- and low-temperature phases, respectively. The same result also follows from the regular solution theory [(Eq. (9)] at low solute concentrations.

A useful simplification of Eq. (10) follows if we neglect the change of the solute concentration in the interfacial water at the phase transition and set $c' = c'' = c_L$:

$$\frac{dT_{tr}}{dc} = \frac{RT_{tr}^2}{\Delta H} (x' - x'') (1 - c_L/c) \qquad (11)$$

The first factors in the right-hand side of Eq. (11) refer to properties of the lipid phases, while the last one, $(1 - c_L/c)$, is solute-specific and defines the solute influence as exerted solely via its uneven distribution between bulk and interlamellar water.

Some general features of the transition temperature behavior can be readily deduced from the above relations:

1. An important result evident from Eqs. (10) and (11) is that the absolute value of dT_{tr}/dc is inversely proportional to the phase transition latent heat ΔH. This feature is identical to the standard Clapeyron-Clausius equation that relates changes in transition temperature to changes in pressure.

2. In the case of diluted solutions, the ratios c'/c and c''/c depend on the pressure and temperature only. Hence, see Eqs. (10) and (11), at small c the transition temperature T_{tr} is a linear function of c.

3. One may define kosmotropic solutes by the inequalities c', $c'' < c$, and chaotropic solutes by the inverse inequalities c', $c'' > c$. This definition is an accord with the experimental observations of solute distribution at interfaces [see, e.g., (Collins and Washabaugh 1985)]. It means that interlamellar water is depleted by kosmotropes, and enriched by chaotropic, in comparison to bulk water. Then, from the simplified version in Eq. (11) it follows that:

a) For kosmotropes the slopes of T_{tr} is positive when the low-temperature phase is the less hydrated one ($x'' < x'$) and negative in the opposite case ($x'' > x'$). In contrast, for chaotropes the slope of T_{tr} is negative when the low-temperature phase is the less hydrated one ($x'' < x'$) and positive in the opposite case ($x'' > x'$).

b) For neutral solutes $c_L = c$ and, consequently, $dT_{tr}/dc = 0$.

c) For phases of equal hydration, $x' = x''$, the transition temperature is not sensitive to solute concentration.

4. It is clear from Eq. (9) that all these conclusions also follow under a regular solution assumption at low solute concentrations.

Discussion

Correlations between experiment and thermodynamic theory

Since the derivation of Eqs. (10) and (11) assumes low solute concentrations, we estimated and summarized in Table 5 the initial slopes of the transition shifts produced by the various solutes. The data available in the literature concerning the lipid phase behavior as affected by the presence of solutes have been also reviewed from the viewpoint of the above relation and summarized in Tables 6 and 7.

The comparison of the experimental results shown in Tables 1–7 and Figs. 1–7 and the thermodynamic description of the solute effects represented by Eq. (11) results in several important conclusions:

Table 6 Effect of different solutes on the phase transition temperatures of hydrated PE

Lipid	Solute	Slope (dT_{tr}/dc) [K/M]		References
		$L_\beta - L_\alpha$	$L_\alpha - H_{II}$	
	Salts			
DMPE	NaCl	1.5	−8.0	Harlos and Eibl 1981
T_m=49.3 °C; ΔH_m=5.8 kcal/mol		(1–4 M)	(3–6 M)	Seddon et al. 1983
T_h=140.0 °C; ΔH_h=0.3 kcal/mol				
DPPE	NaCl	2.0	−7.4	Harlos and Eibl 1981
T_m=62.3 °C; ΔH_m=8.3 kcal/mol	$CaCl_2$	3.4		Doerfler et al. 1990
T_h=130.0 °C; ΔH_h=0.5 kcal/mol				
POPE	Na_2SO_4		−16.0	Sanderson et al. 1991
T_m=24.4 °C; ΔH_m=4.5 kcal/mol	NaCl		−7.3	Sanderson et al. 1991
T_h=70.8 °C; ΔH_h=0.3 kcal/mol	NaBr		−3.5	Sanderson et al. 1991
	NaI		6.0	Sanderson et al. 1991
	GuHCl		8.0	Sanderson et al. 1991
	NaSCN		27.0	Sanderson et al. 1991
DSPE	NaCl	1.3	−4.3	Harlos and Eibl 1981
T_m=73.7 °C; ΔH_m=9.1 kcal/mol				
T_h=101.8 °C; ΔH_h=0.8 kcal/mol				
DEPE	Na_2SO_4	1.7	−9.9	Epand and Bryszewska 1988
T_m=37.3 °C; ΔH_m=6.6 kcal/mol	NaOAc	1.5	−9.1	Epand and Bryszewska 1988
T_h=62.2 °C; ΔH_h=0.6 kcal/mol	NaCl	1.3	−5.6	Epand and Bryszewska 1988
	NaSCN	−3.3	20.0	Epand and Bryszewska 1988
	GuHCl	−4.6	5.0	Epand and Bryszewska 1988
	GuHSCN	−6.5	50.0	Epand and Bryszewska 1988
DTPE	NaCl	1.2	−3.8	Seddon et al. 1983
T_m=55.2 °C; ΔH_m=5.7 kcal/mol				
T_h=96.0 °C; ΔH_h=0.7 kcal/mol				
DHPE	NaCl	1.3	−2.5	Seddon et al. 1983
T_m=68.7 °C; ΔH_m=9.9 kcal/mol				
T_h=85.0 °C; ΔH_h=1.2 kcal/mol				
	Monosaccharides			
DEPE	Glucose		−0.2	Bryszewska and Epand 1988
	Galactose		−0.6	Bryszewska and Epand 1988
	Fructose		−2.5	Bryszewska and Epand 1988
	Sugar alcohols			
POPE	Sorbitol		−9.0	Sanderson et al. 1991
DEPE	Sorbitol		−5.4	Bryszewska and Epand 1988
	myo-Inositol		−5.5	Bryszewska and Epand 1988
	Other polyols			
POPE	Glycerol		−3.3	Sanderson et al. 1991
DSPE	Glycerol	0.9	−1.0	Williams et al. 1991
DEPE	Glycerol		−2.8	Bryszewska and Epand 1988
	PEG		0.2	Bryszewska and Epand 1988
	Disaccharides			
POPE	Sucrose		−16.5	Sanderson et al. 1991
	Raffinose		−30.0	Sanderson et al. 1991
DEPE	Sucrose		−12.6	Bryszewska and Epand 1988
	Trehalose		−10.8	Bryszewska and Epand 1988
	Lactose		−10.0	Bryszewska and Epand 1988
	Maltose		−9.0	Bryszewska and Epand 1988
DMPE	Sucrose	0.3		Cevc 1988
DSPE	Sucrose	1.4	−8.4	Koynova et al. 1989
	Trehalose	1.1	−8.7	Koynova et al. 1989
	Amino acids			
DHPE	Proline	0.9	−1.8	Tsvetkova et al. 1991
	Others			
POPE	Urea		1.3	Sanderson et al. 1991

Table 7 Effect of salts on the phase transition temperatures of hydrated PC

Lipid	Solute	Slope (dT_{tr}/dc) [K/M]		References
		Pre-transition	Main transition	
DLPC				
T_p=−16.0 °C	NaCl	1.33	0.33	Cevc 1991
T_m=0.0 °C; ΔH_m=4.1 kcal/mol				
DMPC				
T_p=14.1 °C; ΔH_p=1.2 kcal/mol	Gu.SCN		−3.8	Chapman et al. 1977
T_m=23.6 °C; ΔH_m=6.0 kcal/mol	KSCN		−2.5[a]	Cunningham et al. 1989
	NaI		−2.4	Chapman et al. 1977
	Gu.Cl	−5.9	−0.4	Chapman et al. 1977
	NaBr	−3.1	0	Chapman et al. 1977
	TlOAc	0.1	0.2	Chapman et al. 1977
	CsCl	−0.5	0.3	Chapman et al. 1977
	KCN	−0.2	0.5	Chapman et al. 1977
	NaCNO	0.4	0.5	Chapman et al. 1977
	KCl	1.4	0.7	Chapman et al. 1977
	$(CH_3)_4N^+Cl^-$	2.0	0.7	Chapman et al. 1977
	NaOAc	3.4	0.9	Chapman et al. 1977
	PbOAc	0.5	1.0	Chapman et al. 1977
	$CdCl_2$	2.8	1.0	Chapman et al. 1977
	NaCl	2.0	1.1	Chapman et al. 1977
	$CuSO_4$	4.2	1.2	Chapman et al. 1977
	NaF	2.7	1.5	Chapman et al. 1977
	$AgNO_3$	3.2	2.0	Chapman et al. 1977
	$MgCl_2$	5.5	2.1	Chapman et al. 1977
	NH_4Cl	3.0	2.6	Chapman et al. 1977
	$CaCl_2$		5.0	Chapman et al. 1977
DPPC				
T_p=35.0 °C; ΔH_p=1.3 kcal/mol	KSCN		0.4[a]	Cunningham et al. 1986a, 1989; Simon et al. 1975
T_m=41.3 °C; ΔH_m=8.2 kcal/mol	$(C_2H_5)_4NBr$		−6.3	Hauser et al. 1977
	$(CH_3)_4N^+Cl^-$		−4.3	Hauser et al. 1977
	KI		−1.3	Simon et al. 1975
	CdOAc		−0.9	Simon et al. 1975
	$CsNO_3$		−0.8	Tolgyesi et al. 1985
	$NaNO_3$		−0.8	Tolgyesi et al. 1985
	$LiNO_3$		−0.5	Tolgyesi et al. 1985
	KNO_3		−0.4	Tolgyesi et al. 1985
	$SrCl_2$		−0.1	Simon et al. 1975
	CsCl	0.0	0.1	Cunningham et al. 1986a; Simon et al. 1975; Sapia and Sportelli 1994; Tolgyesi et al. 1985
	RbCl	0.7	0.1	Tolgyesi et al. 1985
	KBr	1.0	0.4	Cunningham et al. 1986a, b; Simon et al. 1975; Tolgyesi et al. 1985
	NaCl	1.4	0.5	Cunningham et al. 1986a; Hauser et al. 1977; Tolgyesi et al. 1985
	$LaCl_3$		0.7	Simon et al. 1975
	LiCl	3.9	0.8	Cunningham et al. 1986a; Simon et al. 1975; Tolgyesi et al. 1985; Dorfler et al. 1990
	$CdSO_4$		1.1	Simon et al. 1975
	NH_4Cl	1.9	1.1	Cunningham et al. 1986a; Cunningham and Lis 1986
	KCl	−1.0	1.1	Cunningham et al. 1986a; Cunningham and Lis 1986
	$Cd(NO_3)_2$		1.3	Simon et al. 1975
	CdI_2	3.5	1.7	Simon et al. 1975
	$MgCl_2$		1.8	Simon et al. 1975
	$BaCl_2$	2.6	1.8	Cunningham et al. 1986a; Simon et al. 1975
	KOAc	1.6	1.9	Cunningham et al. 1986a; Simon et al. 1975
	$CaCl_2$		3.5	Simon et al. 1975
	$FeCl_3$		7.5	Simon et al. 1975
	Ca^{2+}		11.7	Mishima et al. 1984
	$CdCl_2$		15.7	Simon et al. 1975
DSPC				
T_p=50.0 °C; ΔH_p=1.4 kcal/mol	KSCN	3.3[b]	2.1[a]	Cunningham et al. 1989
T_m=54.0 °C; ΔH_m=10.4 kcal/mol	$NaClO_4$		−2.0	Mendelsohn and Van Holten 1979
	NaCl	2.0	3.0	Cevc 1991
DEPC				
T_m=10.0 °C; ΔH_m=7.8 kcal/mol	$MnCl_2$		4.0	Epand and Bryszewska 1988

[a] $L_\beta^{int}-L_\alpha$ transition (Cunningham et al. 1989) [b] $L_c-L_\beta^{int}$ transition (Cunningham et al. 1989)

(1) Transitions of smaller latent heat such as $L_\alpha \rightarrow H_{II}$ and $L_{\beta'} \rightarrow P_{\beta'}$ are more sensitive to solutes than transitions of larger latent heat such as the chain-melting transitions. This effect is a direct consequence of the inverse proportionality between dT_{tr}/dc and ΔH in Eq. (11).

(2) The experimentally observed linear change of the transition temperature at small solute concentration ($\Delta T_{tr} \sim \Delta c$) follows from Eq. (11) under the assumption of dilute (ideal) solutions. The deviations from linearity at higher solute concentrations (Figs. 2 and 3B, 4A, 6A) might indicate that the ideal solution assumption is not valid at these concentrations.

(3) According to Eq. (11), the opposite effects of kosmotropic and chaotropic solutes are due to their uneven distribution between bulk and interlamellar water. Kosmotropes tend to be excluded from the interface ($c_L < c$), while chaotropes tend to accumulate at the interface ($c_L > c$). The property of Hofmeister solutes to unevenly distribute between bulk water and interfacial water is well known from a large number of studies (e.g., Collins and Washabaugh 1985; Arakawa and Timasheff 1984; Epand and Bryszewska 1988). Thus, Eq. (11) correctly predicts the sign of dT_{tr}/dc in the different transitions. For example, chain-melting transitions move upwards in the presence of kosmotropic solutes because both terms on the right side of Eq. (11), $(1-c_L/c)$ and $(x'-x'')$, are positive in this case. A similar argument shows that chaotropes must induce an upward shift of the $L_\alpha \rightarrow H_{II}$ transitions, as observed experimentally.

(4) Compared to the L_β and H_{II} phases, the lamellar liquid-crystalline phase L_α has the maximum surface area per liquid molecule (Seddon et al. 1984). Since kosmotropes are excluded from the interfacial water and therefore favor minimization of the lipid area exposed to water, they tend to destabilize the L_α phase and to reduce its existence range. In contrast, chaotropic solutes tend to expand the lipid interface area and the stability range of the L_α phase. These considerations are in complete agreement with the predictions of Eq. (11). The scarce data about water contents of the various lipid phases, given by x' and x'' in Eqs. (10) and (11), and also about solute distributions, defined by c_L/c in Eq. (11), preclude any detailed quantitative comparisons between experimentally determined transition shifts and those calculated from Eq. (11).

Phosphatidylethanolamines

Best studied at present are the effects of different solutes on the phase behavior of phosphatidylethanolamines, presumably due to their rich polymorphism, including a variety of lamellar and non-lamellar phases. The solutes used in various studies include inorganic salts, mono- and disaccharides, sugar alcohols, some polyolys, amino acids, urea, etc. The published data are summarized in Table 6, with the initial slopes of the transition shifts caused by solutes, dT_{tr}/dc, calculated.

As evident from the data shown in Table 5, as well as from the results of other studies, summarized in Table 6, the solute effects on the phosphatidylethanolamine phase behavior obey the general rules following from Eqs. (10) and (11) as outlined in the previous section.

The solute-induced temperature shifts of the $L_\beta \rightarrow L_\alpha$ and $L_\alpha \rightarrow H_{II}$ transitions in phosphatidylethanolamines are of opposite sign, i.e., an upward shift of one of these two transitions is always combined with a downward shift of the other transition. Chaotropic solutes (NaI, Gu.HCl, Gu.HSCN, NaSCN, urea) induce an upward shift of the $L_\alpha \rightarrow H_{II}$ transition, while kosmotropic solutes (sugars, sugar alcohols, polyols, proline) induce a downward shift of this transition. At the same time, the $L_\beta \rightarrow L_\alpha$ transition moves in the opposite direction. On average, the temperature shifts of the latter transition are smaller than those of the $L_\alpha \rightarrow H_{II}$ transition.

A remarkable manifestation of the solute effect is that high enough concentrations of sucrose or NaCl fully suppress the L_α phase in some lipids (Fig. 1; Table 6). Consequently, direct $L_\beta \rightarrow H_{II}$ transitions take place in dihexadecylphosphatidylethanolamine and distearoylphosphatidylethanolamine under these conditions. In dimyristoylphosphatidylethanolamine, high NaCl concentration induces formation of an H_{II} phase, which does not appear in pure water (Seddon et al. 1983). In contrast, 1 M NaSCN eliminates the H_{II} phase in soybean phosphatidylethanolamine (Yeagle and Sen 1986).

According to their effect on the $L_\alpha \rightarrow H_{II}$ transition of palmitoyloleoylphosphatidylethanolamine and dielaidoylphosphatidylethanolamine (Table 6), the sodium salts arrange as follows:

$$SO_4^{2-} > OAc^- > Cl^- > Br^- > I^- > SCN^- \qquad (12)$$

This is a characteristic lyotropic (Hofmeister) series for anions. It can be also obtained by many other methods, for example, by the order in which these anions elute from a Sephadex G-10 column (Collins and Washabaugh 1985). The coincidence of the lyotropic series determined here from the magnitude of the transition shifts with those determined by other methods for other systems shows an identical ordering in the anion distribution at the lipid-water and at various other interfaces. However, the sign inversion point between "kosmotropic" and "chaotropic" sides of the lyotropic series for phosphatidylethanolamines (12) is between Br^- and I^-, while this point is found to be about the position of the chloride anion in other systems (for example, Sephadex column, protein solutions, etc.) (Collins and Washabaugh 1985).

In Table 1, the effects of sucrose and NaSCN on other thermodynamic parameters, calorimetric enthalpy ΔH and transition half-width $\Delta T_{1/2}$, in the case of DHPE are also accounted for. It is clear from the table that these effects are not as distinct as the solute effect on the transition temperature. Sucrose appears to influence in opposite directions the enthalpies of the $L_\beta \rightarrow L_\alpha$ and $L_\alpha \rightarrow H_{II}$ transitions, while NaSCN considerably broadens both transitions and reduces the enthalpy of the $L_\alpha \rightarrow H_{II}$ transition. As these effects become apparent at rather high solute concentrations, we assume that they may reflect direct interactions with the lipid interface or incorporation of solute

molecules into the hydrophobic core of the lipid aggregates. It is appropriate to note in this connection that the derivation of Eqs. (10) and (11) assumes a first-order, infinitely narrow transition of a given enthalpy between the adjacent phases. These equations are therefore irrelevant to solute effects on the transition width and enthalpy.

Phosphatidylcholines

Solute effects have been studied mainly for the saturated diacyl phosphatidylcholines of equal chain length of 14, 16 and 18 carbon atoms. These are bilayer-forming lipids characterized by the phase sequence:

$$L_c \rightarrow L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_\alpha \qquad (13)$$

Solute effects have been followed for the pretransition ($L_{\beta'} \rightarrow P_{\beta'}$) and the main transition ($P_{\beta'} \rightarrow L_\alpha$), mainly in dipalmitoylphosphatidylcholine (Rudolph and Goins 1991; Tolgyesi et al. 1985; Lis et al. 1990; Yamazaki et al. 1992a, b; Cunningham et al. 1986a, b, 1989; Simon et al. 1975; Hauser et al. 1977; Mishima et al. 1984; Dorfler et al. 1990; Bartucci and Sportelli 1993; Sapia and Sportelli 1994), and also in dimyristoylphosphatidylcholine (Chapman et al. 1977; Cunningham et al. 1989) and distearoylphosphatidylcholine (Cevc 1991; Mendelsohn and Van Holten 1979; Cunningham et al. 1989). Here we also report results concerning the $L_c \rightarrow L_{\beta'}$ transition of dipalmitoylphosphatidylcholine in proline and sucrose solutions (Table 4). Single studies report the effect of NaCl on the pre- and main transition of DLPC (Cevc 1991) and that of $MnCl_2$ on the melting transition of DEPC (Epand and Bryszewska 1988).

Both literature data and the results with proline and sucrose reported here show that the pretransition and main transition generally move in the same direction with the solute concentration, and that the former transition is more sensitive to solutes than the latter one. As with the chain-melting transition in phosphatidylethanolamines, the main transition in DPPC shifts to higher temperatures in the presence of kosmotropic solutes. A noteworthy consequence of the greater sensitivity of the pretransition to solutes is that it merges with the main transition at high enough solute concentrations. Sucrose and proline at concentrations of 2.4 M and 3 M, respectively, fully suppress the rippled $P_{\beta'}$ phase, as demonstrated in Fig. 2. As noted above, we consider the greater sensitivity of the pretransition, the suppression of the rippled phase by kosmotropic solutes, to be a consequence of its much lower enthalpy compared to that of the main transition.

The data available in the literature concerning the effect of different salts on the phosphatidylcholine phase transition temperatures are reviewed and summarized, and the initial transition shifts ΔT_{tr} for 1 M solutes for saturated diacyl phosphatidylcholines with hydrocarbon chains 12–18 carbon atoms long, are calculated in Table 7. On basis of their effect on the $L_{\beta'} \rightarrow P_{\beta'}$ and $P_{\beta'} \rightarrow L_\alpha$ transitions of dipalmitoylphosphatidylcholine, the alkali chlorides arrange in the order:

$$Li^+ > Na^+ > K^+ > Rb^+ > Cs^+ \qquad (14)$$

Identical Hofmeister series for cations have been obtained by numerous other experiments (chromatographic behavior, ionic entropy of dilution, etc.) (Collins and Washabaugh 1985). According to the data in Table 7, the kosmotropic/chaotropic sign inversion point for the cation series (14) in the case of dipalmitoylphosphatidylcholine is at the position of Cs^+. In other systems, this point was found to be near the position of Na^+ (Collins and Washabaugh 1985). A comparison of Tables 6 and 7 shows that the anion (12) modify the chain-melting temperature of the lipids in a much broader range than the cation series (14).

According to published data, the enthalpy of the dipalmitoylphosphatidylcholine $L_{\beta'} \rightarrow P_{\beta'}$ and $P_{\beta'} \rightarrow L_\alpha$ transitions is not sensitive to the addition of solutes (Tolgyesi et al. 1985; Lis et al. 1990).

Ethylene glycol, its dimers, trimers, and polymers very slightly modulate the phase behavior of dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine (Yamazaki et al. 1992a, b). These data suggest that ethylene glycol and di-ethylene glycol might be classified as weak chaotropes, while triethylene glycol and polyethylene glycol appear to act as weak kosmotropes.

Glycolipids

Our results concerning the transition shifts of synthetic ether-linked glycoglycerolipids with saturated chains of equal length as given in Table 3 show that the solute effects in glycolipids are governed by the same general rules as in the case of phosphatidylethanolamines – chaotropic solutes support formation of L_α at the expense of L_β and H_{II}, while kosmotropic solutes reduce the temperature range of the L_α phase. For example, 0.3 M trehalose eliminates the L_α phase and brings about a direct $L_\beta \rightarrow H_{II}$ transformation in ditetradecylglucosylglycerol (Fig. 3). On the other hand, high NaSCN concentrations suppress the formation of the H_{II} phase. A noteworthy effect of the chaotropic solute NaSCN is the induction of intermediate L_α and/or cubic phases in 16-Glc and 16-Man, undergoing direct $L_\beta \rightarrow H_{II}$ transitions in pure water. These two glycolipids have very similar chemical structure and phase behavior in pure water. It is interesting to note in this connection their different sensitivity to NaSCN. The direct $L_\beta \rightarrow H_{II}$ transition splits into an $L_\beta \rightarrow L_\alpha/Q \rightarrow H_{II}$ sequence at about 2.5 M NaSCN in 16-Man, while in 16-Glc the splitting of this transition takes place immediately upon addition of NaSCN. The effect of NaSCN on the $L_\alpha \rightarrow H_{II}$ transitions in glycolipids is much smaller than the corresponding effect in phosphatidylethanolamines (compares Tables 2 and 3).

Conclusions

It is clear from the present and reviewed data that, according to their effect on the lipid phase transitions, the low-

molecular Hofmeister solutes fall into two categories: i) solutes favoring formation of the lamellar liquid crystalline phase L_α at the expense of the L_β and H_{II} phases; ii) solutes favoring formation of L_β and H_{II} at the expense of the L_α phase. These effects are correctly described by an equation of the Clapeyron-Clausius type, derived in the present work as a relation between phase transition temperature and solute concentration. It shows that the solute effect, dT_{tr}/dc, is proportional to the hydration difference between the two lipid phases and inversely proportional to the latent heat of the phase transition. The sign and magnitude of the transition shifts induced by the different solutes depend on the solute's ability to unevenly distribute between interlamellar and free water. A detailed quantitative application of this relation is prevented, however, by the lack of data about water contents of the coexisting lipid phases and about solute distributions between interlamellar and bulk water.

Kosmotropic solutes tend to minimize the area of the lipid-water contact. They suppress the L_α phase, as it has the largest surface area in contact with water. At high enough concentration of kosmotropic solutes, this phase may completely disappear from the phase diagram. This is precisely what is seen with sucrose, trehalose, proline and some salts, and is consistent with the opposite effect caused by chaotropic solutes. Moreover, addition of chaotropic solutes can induce the appearance of the missing liquid-crystalline phases. Our calorimetric studies on glycolipids have shown that the denaturing agent NaSCN converts the direct $L_\beta \rightarrow H_{II}$ transitions into $L_\beta \rightarrow L_\alpha/Q \rightarrow H_{II}$ sequences with stable L_α/Q intermediates appearing at the expense of the L_β and H_{II} phases.

In comparison with other measures of the Hofmeister series with inorganic salts, the lipid phase transition temperatures give identical anion and cation rank ordering. However, the sign inversion point, for both anions and cations, is located in the "chaotropic" side of the series. Another noteworthy feature is that anions have a greater effect on the transition temperature than cations.

To our knowledge, no systematic studies on the effects of chaotropic and kosmotropic solutes have been carried out for lipid-water systems forming cubic phases and also for lyotropic systems other than those formed by membrane lipids. Such studies can be expected to result in methods for regulation of the phase behavior of lyotropic liquid crystals in a broad range of temperatures by controlling the composition of the aqueous phase. It is noteworthy with regard to this goal that the experiments with membrane lipids have shown that the boundaries between lamellar and non-lamellar phases can be shifted by several tens of degrees in either direction, merely by addition of appropriate chaotropic or kosmotropic solutes to the aqueous phase.

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