

LAMELLAR-TO-INVERTED HEXAGONAL PHASE TRANSITION IN LYOTROPIC LIQUID CRYSTALS IN THE PRESENCE OF HOFMEISTER SOLUTES

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ABSTRACT: The Hofmeister effect refers to the effects of ions and small non-ionic solutes, termed Hofmeister solutes, on a wide range of phenomena in aqueous milieu, such as colloid stability, critical micelle concentration, emulsion microstructure, cloud points of polymer solutions, zeta potentials, ion binding to interfaces, phase transitions in lipid dispersions and many other. Using an equation of Clapeyron-Clausius type, derived by our late colleague Professor Jordan G. Brankov in our previous study on the Hofmeister effect (*European Biophysics Journal* (1997) **25** 261-274), here we analyze the relation of the lipid headgroup hydration to the effect of Hofmeister solutes on the lamellar-to-inverted hexagonal phase transition in aqueous lipid dispersions and develop a new approach for determination of the headgroup hydration difference between the lamellar and inverted hexagonal phases.

KEY WORDS: Hofmeister effect, lyotropic series, lyotropic liquid crystals, lipids, lamellar phase, inverted hexagonal phase, phase transition, Clapeyron-Clausius equation.

ABBREVIATIONS

PE : phosphatidylethanolamine
DPPE : dipalmitoyl PE
DPoPE: dipalmitoleoyl PE
DOPE : dioleoyl PE
16-Glc: dihexadecyl glucosylglycerol
DSC : differential scanning calorimetry

DHPE : dihexadecyl PE
DSPE : distearoyl PE
POPE : palmitoyl oleoyl PE
14-Glc : ditetradecyl glucosylglycerol
16-Man: dihexadecyl mannosylglycerol

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1 LAMELLAR-TO-INVERTED HEXAGONAL PHASE TRANSITION IN AQUEOUS LIPID DISPERSIONS

Numerous surfactants, including a large group of lipid molecules present in the biological membranes, are known to form a variety of liquid crystalline phases in aqueous dispersions. The inherent ability of these molecules to form lyotropic liquid crystals is a direct consequence of their amphiphilic structure, comprising a hydrophobic component (typically two hydrocarbon chains in the lipid molecules) and a polar group that interacts with the aqueous phase and prevents the direct contacts of the surfactant hydrophobic parts with the water molecules. These molecules can form several lyotropic liquid crystalline phases of different structure and geometry. In our previous research, carried out in collaboration with Professor Jordan G. Brankov, we investigated both experimentally, by differential scanning calorimetry (DSC) and by X-ray diffraction, and theoretically, in the framework of a thermodynamic approach based on an equation of Clapeyron-Clausius type, the effects of various solutes on the positions of the phase boundaries between the lipid phases formed in excess water dispersions [1]. A most interesting outcome of that study was that the observed effects as well as their thermodynamic description represent a typical, clear-cut manifestation of the long-known but still poorly understood Hofmeister effect [2].

In the present work we apply the same methodology and focus on the phase transition between the liquid crystalline lamellar L_α phase and the inverted hexagonal H_{II} phase in lipid dispersions, in particular, on the relation between the $L_\alpha \leftrightarrow H_{II}$ phase transition temperature and the solute concentration.

The lamellar liquid crystalline L_α phase is a frequently occurring phase, studied in significant detail. It can be formed by virtually all lipid types present in the biological membranes, except for cholesterol. The L_α phase represents a stack of parallel liquid crystalline lipid bilayers, separated by aqueous layers of thickness w (Fig. 1, left).

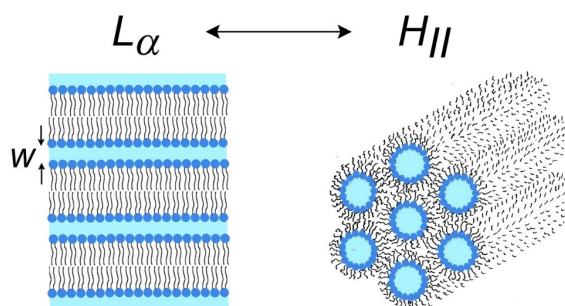


Fig. 1: Structures of the lamellar phase L_α (left) and the inverted hexagonal phase H_{II} (right) forming in aqueous dispersions of biomembrane lipids.

The properties of the L_α phase are of particular interest because the liquid crystalline lipid bilayer is the fundamental structural unit of the biological membranes. Another liquid crystalline phase of significant interest is the inverted hexagonal phase, H_{II} , which is made of hexagonally packed cylinders with internal, filled by water cylindrical spaces (Fig. 1, right). Many biomembrane lipids can form an H_{II} phase, including various glycolipids and the phosphatidylethanolamines (PEs), which are the second most abundant phospholipid class in the biomembranes after the phosphatidylcholines. The H_{II} phase appears at higher temperatures than the L_α phase and, whenever present, it limits from above the temperature existence range of the L_α phase. The transition from L_α phase to H_{II} phase, taking place with increase of temperature, is a reversible, highly cooperative, presumably first-order phase transition. It is readily detected by DSC as a sharp, narrow heat capacity peak with the peak area representing the latent heat (enthalpy) of the transition (Figs. 2A and 3A). The $L_\alpha \leftrightarrow H_{II}$ transition is typified by a clear-cut two-phase co-existence of the L_α and H_{II} phases, accompanied by no other intermediate structures detectable by high-resolution synchrotron X-ray diffraction in the transition region (Figs. 2B and 3B).

The mechanism of the $L_\alpha \leftrightarrow H_{II}$ phase transition has been the subject of numerous experimental and theoretical studies (see e.g. [6–14]). The theoretical modeling

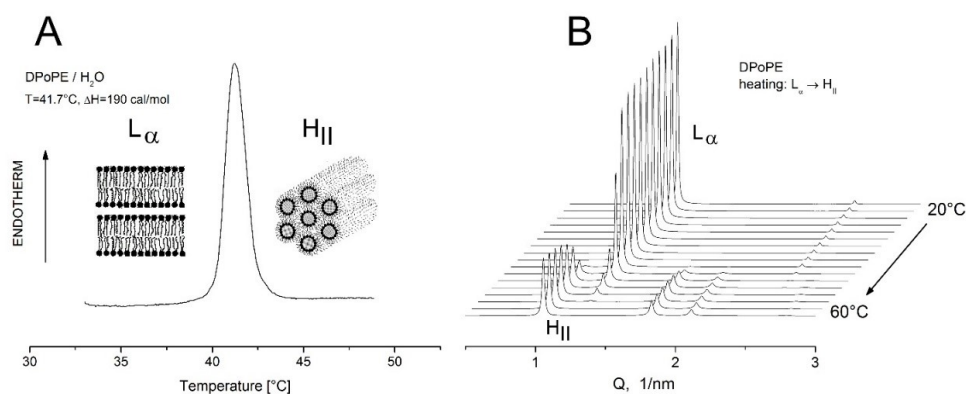


Fig. 2: (A) Heat capacity peak of the $L_\alpha \leftrightarrow H_{II}$ phases transition in dipalmitoleoyl phosphatidylethanolamine (DPOPE) dispersions, recorded by DSC [3]. (B) Consecutive X-ray diffraction profiles recorded by time-resolved X-ray diffraction that demonstrate co-existence of the L_α and H_{II} phases during a heating scan through the $L_\alpha \leftrightarrow H_{II}$ phase transition in DPOPE dispersions. The heating rates in (A) and (B) were 1 K/min (adapted from [4]).

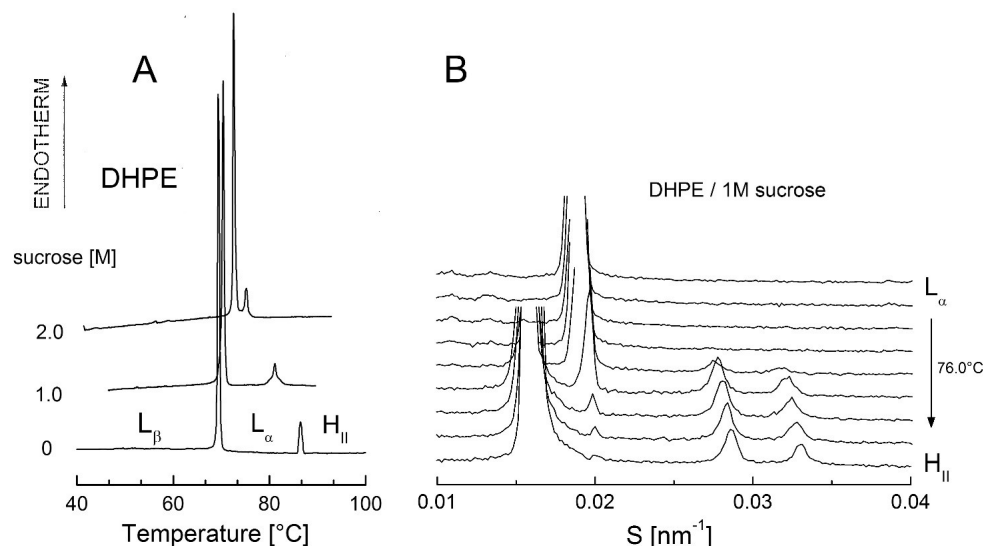


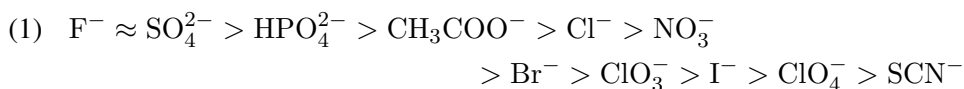
Fig. 3: (A) Heat capacity peaks of the gel-liquid crystal $L_\beta \leftrightarrow L_\alpha$ phase transition and the $L_\alpha \leftrightarrow H_{II}$ phase transition in dihexadecyl PE (DHPE) dispersions in excess water at 0, 1 and 2 M sucrose concentrations, recorded by DSC [1]. (B) Consecutive X-ray diffraction profiles recorded by time-resolved X-ray diffraction that demonstrate co-existence of the L_α and H_{II} phases during a heating temperature scan through the $L_\alpha \leftrightarrow H_{II}$ phase transition in DHPE dispersions at 1M sucrose concentration [5]. The heating rates in (A) and (B) were 1 K/min.

of the $L_\alpha \leftrightarrow H_{II}$ phase transition aims to account for the energetics of the prominent structural distinctions between the two phases, such as flat lipid monolayers in the L_α phase vs. cylindrically curved lipid monolayers with inward pointing polar head groups in the H_{II} phase. To our knowledge, no attempts have so far been made to integrate the effects of the Hofmeister solutes in the framework of the published theoretical models. However, debating this topic is beyond the scope of this article and we will return to it in a subsequent publication.

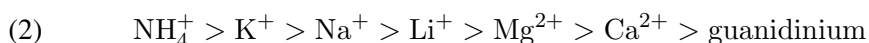
2 THE HOFMEISTER EFFECT ON THE PHASE TRANSITIONS IN AQUEOUS LIPID DISPERSIONS

Although the Hofmeister effect was discovered more than 130 years ago [2], its nature still remains obscure [15, 16]. Hofmeister found that salts order in series according to their effect on the protein solubility and stability of the protein spatial (secondary

and tertiary) structure. Anions appear to have a larger effect than cations and usually follow the order



Cations usually order as follows:



Equations 1 and 2 only provide partial listings of Hofmeister series for illustrative purposes. Many more salts have actually been studied in the long history of studies on the Hofmeister effect. These series start on the left-hand side with kosmotropic, structure making solutes, such as fluoride, sulfate and phosphate, and end on the right-hand side with chaotropic, structure-breaking solutes, such as thiocyanate and guanidinium. Although its mechanism is not entirely clear, the Hofmeister effect seems to result mainly from the ordering or disordering of the solvent (water) structure by kosmotropic, respectively, chaotropic solutes. These effects increase with the solute concentration and become clearly expressed at higher salt concentrations (> 100 mM). Kosmotropic members of the series increase surface tension, decrease the solubility of nonpolar molecules (salting out effect), and stabilize proteins. By contrast, chaotropic solutes decrease surface tension, increase the solubility of non-polar molecules (salting in effect) and destabilize proteins. In effect, the chaotropes weaken the hydrophobic effect.

Colligative properties of solutions are properties that depend upon the concentration of solute molecules or ions, but not upon the identity of the solute. Colligative properties typically include vapor pressure lowering, boiling point elevation, freezing point depression, and osmotic pressure. By contrast, the Hofmeister solutes display effects that depend not only on the concentration, but also on the type of solute. For that reason, the treatments of the Hofmeister effect have met with significant hurdles as they need to account for the individual properties of the Hofmeister solutes in order to clarify the mechanisms of their non-colligative effects.

In biological systems, a large variety of ions exist – from simple ions, such as halides and alkali metals, to complex organic molecules. Ion-specific effects in biological systems were first ordered based on the ability of salts to precipitate egg-white proteins from aqueous solution [2]. Later experiments examining the effect of large variety of different salts on biomolecular systems demonstrated the general validity of the observed sequence, and ions were arranged in two series, one for anions (Eq. 1) and one for cations (Eq. 2), called Hofmeister or lyotropic series. For halide anions, their power to destabilize proteins increases in a sequence from smaller to larger ion

sizes, $F^- < Cl^- < Br^- < I^-$. For alkali metal cations, the sequence follows the opposite order, from larger to smaller ion sizes, $K^+ > Na^+ > Li^+$.

The interest in Hofmeister effects manifested in biological systems is growing in the recent years. The effect of electrolytes and buffer ions on the structure and functionality of proteins, enzymes, biomacromolecules, nucleic acids, biomembranes, cytoplasmatic organelles, and other supramolecular assemblies is certainly a key area for development in science. The further development and elaboration of physico-chemical concepts such as non-electrostatic interactions and hydration can help to solve the complex mechanisms through which ionic species participate and modulate the processes explored by life sciences.

Lipids constitute a diverse group of important biomolecules. In presence of water, they self-assemble in a variety of phases with different structure and geometry, transforming into each other via different types of phase transitions [17]. The lamellar gel-to-lamellar liquid-crystalline ($L_\beta \leftrightarrow L_\alpha$) phase transition is a major energetic event in the lipid bilayers that takes place with a large enthalpy change, much larger than that for the $L_\alpha \leftrightarrow H_{II}$ phase transition (Fig. 3A). It is associated with rotameric disordering of the hydrocarbon chains and increased headgroup hydration. At higher temperatures, the lipid dispersions may display lamellar-to-nonlamellar phase transitions, most often from the lamellar to the inverted hexagonal phase ($L_\alpha \leftrightarrow H_{II}$). In some instances, lipids also form inverted phases of cubic symmetry, Q_{II} . A generalized phase sequence of thermotropic phase transitions in biomembrane lipids may be written as $L_\beta \leftrightarrow L_\alpha \leftrightarrow Q_{II} \leftrightarrow H_{II}$ [17].

The interactions of the lipid polar groups with water make an essential contribution to the energy balance of a given phase. Many lipid phase transitions take place with large changes in the lipid surface area and therefore in the amount of bound water. The lipid hydration is controlled by the chemical structure of the polar groups. However, in fully hydrated systems, with water in excess, the extent of the polar group hydration also depends on the state of bulk water. Various low-molecular solutes are known for their ability to strongly modulate the bulk water structure – “water-structure makers” (kosmotropes) and “water-structure breakers” (chaotropes). This way, it turns out that, even without direct interaction with the lipid polar heads, solutes can largely modulate the properties of the lipid-water interface and therefore the lipid phase behavior. Changes in the aqueous phase composition can shift substantially the temperature ranges of stability of the lipid phases and induce or suppress the formation of certain phases. Such indirect solute effects on the interfacial properties, generally termed the Hofmeister effect, have been found in many lipid-water phases [1, 18–20]. Studies on the nature of the Hofmeister effect indicate that it results from an interplay of electrostatics, dispersion forces, thermal motion, fluctuations, hydration, polarizability, ion size effects, and the impact of interfacial

water [21,22]. According to their effect on the lipid phase transitions, the Hofmeister solutes fall into two categories: (i) chaotropic solutes that favor the formation of the lamellar liquid crystalline phase L_α over the neighboring H_{II} and L_β phases and (ii) kosmotropic solutes that favor the formation of the H_{II} and L_β phase at the expense of the L_α phase. Their effects have been correctly described by an equation of the Clapeyron–Clausius type between phase transition temperature and solute concentration, derived in our previous work [1]. The sign and magnitude of the phase transition shifts induced by the different solutes depend on the solute ability to distribute unevenly between interfacial, lipid-bound water and bulk water (Table 1). 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 [1, 18]. An example is presented in Fig. 3A, with sucrose narrowing the temperature range of the L_α phase stability in DHPE. In Fig. 4, the temperature range of the L_α phase stability is reduced by sucrose in a series of phosphatidylethanolamines (PEs): DHPE, DPPE, DSPE, and POPE. Upon increasing the kosmotropic solute concentration, the latter phase may completely disappear from the phase diagram, as it happens with DSPE [1, 23] and the glycolipid 14-Glc at high sucrose concentrations [1, 18]. Disappearance of the L_α phase from the lipid phase diagrams can be also induced by high concentrations of trehalose, proline, glycerol [23–26] and some salts. It is consistent with the opposite effect caused by chaotropic solutes (Fig. 5; Table 1). The addition of chaotropic

Table 1: Effects of kosmotropes (sucrose, trehalose, proline, glycerol) and chaotropes (NaSCN, GuHCl) on the phase transition temperature slope dT_{tr}/dc of the $L_\alpha \leftrightarrow H_{II}$ phase transitions for a variety of membrane PEs and glycolipids [1, 27, 28]

Solute	Lipid	Slope (dT_{tr}/dc) [K/M] $L_\alpha \leftrightarrow H_{II}$	Solute	Lipid	Slope (dT_{tr}/dc) [K/M] $L_\alpha \leftrightarrow H_{II}$
Sucrose	DHPE	-10.4	Glycerol	DSPE	-0.6
	DPPE	-19.6		POPE	-3.3
	DSPE	-8.8		DEPE	-2.8
	POPE	-18.2	NaSCN	DHPE	4.8
	14-Glc	-3.8		POPE	27.0
Trehalose	DHPE	-11.0		14-Glc	4.4
	DSPE	-8.5		16-Glc	1.6
	14-Glc	-10.7		16-Man	1.0
Proline	DHPE	-1.6	GuHCl	DHPE	3.2
				POPE	8.0

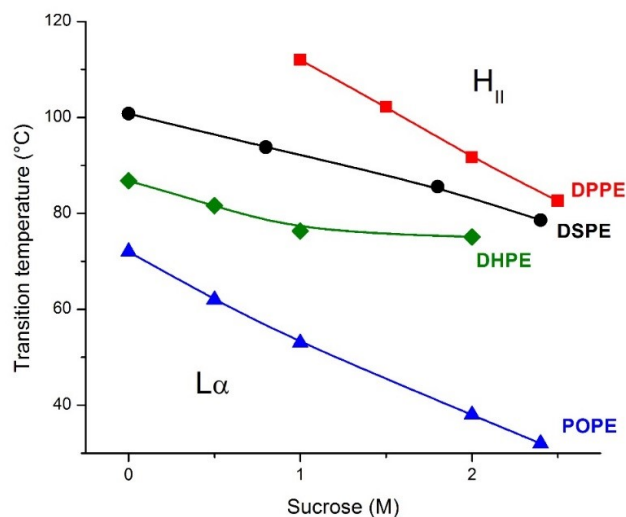


Fig. 4: Phase transition temperatures of the lamellar-to-inverted hexagonal $L_{\alpha} \leftrightarrow H_{II}$ phase transition in phosphatidylethanolamine (PE) dispersions as a function of sucrose concentration [1,27].

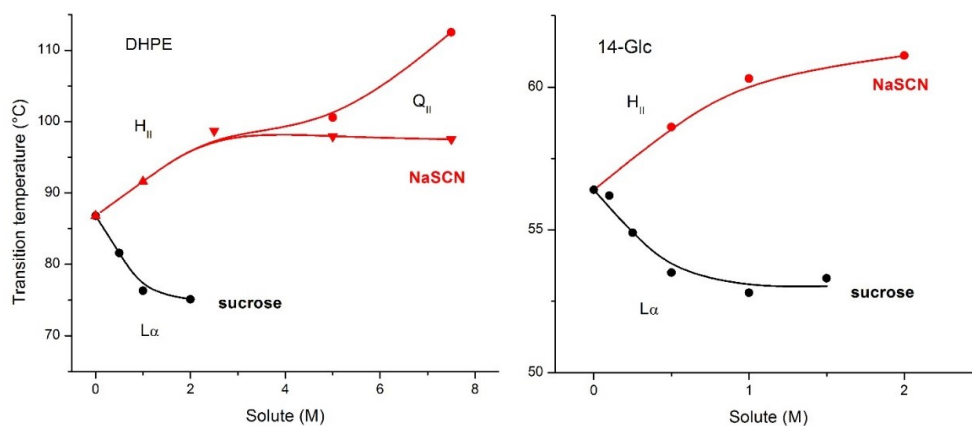


Fig. 5: $L_{\alpha} \leftrightarrow H_{II}$ phase transition temperatures of dihexadecyl phosphatidylethanolamine (DHPE) (left panel), and ditetradecyl glucosylglycerol (14-Glc) (right panel) dispersions in sucrose (black) and NaSCN (red) solutions [1]. Noteworthy, along with the strong upward shift of the $L_{\alpha} \leftrightarrow H_{II}$ phase transition temperature, NaSCN also induced the appearance of an inverted cubic phase Q_{II} between the L_{α} and H_{II} phases in DHPE dispersions at high NaSCN concentrations [5].

solutes can also induce the appearance of the missing liquid-crystalline phases from the general lipid phase sequence. One such example is the appearance of an inverted cubic phase in the DHPE sequence at high concentrations of the chaotropic solute NaSCN (Fig. 5) [1,5].

3 THERMODYNAMIC THEORY OF THE HOFMEISTER EFFECT. CLAPEYRON-CLAUSIUS EQUATION

The lipid-water systems described above are two-phase, three-component systems. The two phases are the lipid phase and a phase of bulk water which is in large excess (reservoir). The three components are lipid, water, and solute molecules. These components unevenly distribute between the two phases. Due to their low solubility, the concentration of lipid molecules (monomers) in the excess water phase is extremely low compared to that in the lipid phase. Besides lipid aggregates, the lipid phase also includes a thin layer of interfacial water bound to the lipid polar headgroups. In general, the solute molecules distribute unevenly between bulk and interfacial water and their uneven distribution is actually the underlying physical cause of the Hofmeister effect in lipid-water systems. At the $L_\alpha \leftrightarrow H_{II}$ phase transition temperatures, the lipid-water system becomes three-phase system with two coexisting lipid phases in equilibrium with the reservoir of excess water. In order to describe the observed Hofmeister solute effects on the phase transition temperature, a general thermodynamic relationship of Clapeyron-Clausius type was derived in our previous work [1]. It relates the change in the transition temperature dT_{tr}/dc to the concentrations of the Hofmeister solutes in the bulk and interfacial water, and to the lipid headgroup hydrations in the L_α and H_{II} phases:

$$(3) \quad \frac{dT_{tr}}{dc} = \frac{RT_{tr}^2}{\Delta H} [(1 - c'/c)x' - (1 - c''/c)x'']$$

Here the prime and second signs denote the high-temperature H_{II} and the low-temperature L_α phases, respectively; ΔH is the transition latent heat (enthalpy) per mol lipid; R is the gas constant; x' and x'' are the numbers of interfacial (bound) water molecules per lipid molecule in each of the two coexisting phases during the phase transition; c' and c'' are solute concentrations in the interfacial water at bulk solute concentration c in the reservoir.

Some general conclusions regarding the Hofmeister solute effects on the $L_\alpha \leftrightarrow H_{II}$ phase transition temperature can be inferred directly from Eq. 3, as is pointed out in [1]. They are as follows:

- It follows from Eq. 3 that ideal neutral solutes, which have identical bulk and interfacial concentrations $c' = c'' = c$, do not have any influence on the transi-

tion temperature dT_{tr}/dc . Respectively, such solutes do not display a Hofmeister effect. However, for kosmotropic and chaotropic solutes the concentrations c' and c'' differ from the bulk concentration c . In that case dT_{tr}/dc . Moreover, its values order in accord with the lyotropic series in a clear-cut manifestation of the Hofmeister effect [1].

- Kosmotropic solutes can be distinguished from chaotropic solutes by the relations $c', c'' < c$ for kosmotropes and $c', c'' > c$ for chaotropes. This definition matches the experimental observations of solute distribution at interfaces which show that interfacial water is depleted in kosmotropic solutes and enriched in chaotropic solutes (see, e.g., ref. [22]).
- In diluted (ideal) solutions, the ratios c'/c and c''/c in Eq. 3 depend only on the pressure and temperature. Hence, at low solute concentrations c , the transition temperature T_{tr} is a linear function of c . This conclusion is confirmed by the experimental DSC data, cf. Figs. 4 and 5.
- The absolute value of dT_{tr}/dc is inversely proportional to the phase transition latent heat ΔH . This feature is matching that in the standard Clapeyron-Clausius equation that relates changes in transition temperature to changes in pressure. As is obvious from Fig. 3A, the latent heat ΔH of the $L_{\alpha} \leftrightarrow H_{\text{II}}$ phase transition is much smaller than the latent heat of the melting solid-fluid $L_{\beta} \leftrightarrow L_{\alpha}$ phase transition. Consequently, the $L_{\alpha} \leftrightarrow H_{\text{II}}$ phase transition temperature is much more sensitive to variations in the Hofmeister solute concentration than the temperature of the $L_{\beta} \leftrightarrow L_{\alpha}$ phase transition, as it has been experimentally verified in our previous work [1].

4 RELATION BETWEEN THE LIPID HYDRATION DIFFERENCE $x^{H_{\text{II}}} - x^{L_{\alpha}}$ AND THE L_{α} TO H_{II} TRANSITION PARAMETERS

Following [1], we assume that $c' = c'' = c_L$ at the transition temperature, where the two phases co-exist. Eq. 3 then becomes

$$(4) \quad \frac{dT_{\text{tr}}}{dc} = \frac{RT_{\text{tr}}^2}{\Delta H} \frac{\Delta c}{c} (x^{H_{\text{II}}} - x^{L_{\alpha}})$$

Here $\Delta c = c - c_L$. For kosmotropic solutes, $c_L < c$ and $\Delta c/c > 0$, whereas for chaotropic solutes the opposite is true, $c_L > c$, respectively, $\Delta c/c < 0$. In addition to its dependence on $\Delta c/c$, the transition temperature derivative dT_{tr}/dc also depends on the sign and magnitude of the hydration difference $x^{H_{\text{II}}} - x^{L_{\alpha}}$ between the two co-existing phases at the transition temperature. Studies on PE hydration have shown that the lipid headgroup hydration decreases at the L_{α} to H_{II} phase transition, i.e.,

the PE headgroups in H_{II} phase bind less water than the same headgroups in L_α phase [29–31]. Based on these findings, the term $x^{H_{II}} - x^{L_\alpha}$ in Eq. 4 turns out to be always negative, making the right-hand side of Eq. 4 negative for kosmotropes and positive for chaotropes. We thus arrive to the conclusion that kosmotropic solutes always depress, while chaotropic solutes always elevate the L_α to H_{II} phase transition temperature, in full accord with the experimental data in Figs. 4, 5 and Table 1. Respectively, the transition slope dT_{tr}/dc may be considered a measure of the sign and magnitude of the Hofmeister effect for the different solutes.

Remarkably, as is evident from Eqs. 3 and 4, the effects of kosmotropes and chaotropes are not symmetric. The kosmotropic effects are limited from above and their maximum is reached at full exclusion of the solute from interfacial water, $c_L = 0$ and $\Delta c/c = 1$, while similar limitations do not exist for chaotropic solutes (in ideal solutions at low solute concentrations). The limiting maximum value of dT_{tr}/dc in the case of kosmotropes is given by Eq. 5

$$(5) \quad \left| \frac{dT_{tr}}{dc} \right|_{\max} = \frac{RT_{tr}^2}{\Delta H} (x^{L_\alpha} - x^{H_{II}}).$$

Using Eq. 5, the reduction in the lipid headgroup hydrations $x^{L_\alpha} - x^{H_{II}}$ upon the L_α to H_{II} phase transition can be expressed as a function of the L_α to H_{II} phase transition parameters. By rearranging Eq. 5, we obtain

$$(6) \quad x^{L_\alpha} - x^{H_{II}} = \frac{\Delta H}{RT_{tr}^2} \left| \frac{dT_{tr}}{dc} \right|_{\max}.$$

Noteworthy, all thermodynamic quantities in Eq. 6 (the phase transition enthalpy ΔH and temperature dT_{tr} , as well as the maximum value of the derivative dT_{tr}/dc) can be readily determined experimentally by DSC. In a first step to check the applicability of Eq. 6 to assess lipid headgroup hydrations, we made numerical estimates, based on Eq. 6, for diester PEs with both saturated and unsaturated hydrocarbon chains (DPPE, DEPE, POPE) that display L_α to H_{II} phase transitions and have been extensively studied by a variety of methods. For the estimates, we used published DSC data about the effect of sucrose (see, e.g., Table 6 in [1] for a summary). Sucrose is known as a strong kosmotrope, thought to be fully excluded from interfacial water [31]. It, therefore, satisfies the precondition $c_L = 0$ required to validate Eq. 6. The estimates showed that the headgroup hydration reductions $x^{L_\alpha} - x^{H_{II}}$ for all three PE lipids were in the range 1.1–1.6 water molecules. These values are very close, within the error limits, to the results obtained using a radiolabel centrifugal technique, which showed headgroup hydration differences between the L_α and H_{II} phases of 1.6–1.8 water molecules for dioleoyl PE and egg PE [31]. The difference between our estimates and published data could be due to the circumstance that the determinations

in [31] were carried out at two different temperatures below and above the L_α to H_{II} phase transition, which are about 12–14°C apart from each other, while our estimates pertain to the same temperature, that is, to the L_α to H_{II} phase transition temperature. The close proximity to data, obtained by a different method, is encouraging and provides grounds to believe that the developed in the present work approach represents a promising and reliable new method for assessment of the amount of bound water.

In this connection, it is worth mentioning that the internal aqueous spaces between the bilayers in the L_α phase and the cylindrical aqueous cavities in the H_{II} phase, as determined by X-ray diffraction [10, 13, 14], contain significantly more water than the bound water amounts associated with the lipid polar headgroups. In particular, the aqueous cylinders of the H_{II} phase were shown to contain more water per lipid molecule than the interlamellar aqueous spaces of the L_α phase [6, 10, 30, 32], opposite to the smaller quantity of bound water in the H_{II} relative to the L_α phase. Based on the published data we are aware of, it appears that, on the average, bound water represents approximately 50% and 30% of the total water content in the L_α phase and the H_{II} phase, respectively.

Although the lipid headgroup hydrations x^{L_α} and $x^{H_{II}}$ cannot be derived solely on basis of the X-ray data about the L_α and H_{II} geometric parameters, the data on the lipid headgroup areas can still be useful, in combination with other methods, in assessments of the headgroup hydration. Noting that the smaller headgroup hydration in the H_{II} phase relative to the L_α phase is likely caused by the reduction of the lipid surface area upon the L_α to H_{II} phase transformation, we may assume, in a first approximation, that the reduction in headgroup hydration is proportional to the surface area reduction, and, accordingly, that the quantity of bound water molecules is proportional to the lipid headgroup areas at the lipid–water interfaces in the L_α and H_{II} phases. An estimate for DOPE, based on the latter assumption, provided values of about 7.7 and 6.1 bound water molecules per lipid molecule for the L_α and H_{II} phases, respectively. These values are close to the analogous values of 7.2 and 5.4 water molecules per DOPE headgroup measured in [31] at temperatures close to the L_α to H_{II} phase transition temperature. However, in view of the simplifications and uncertainties involved, the last estimate should be considered as an illustration only that would need substantial additional verification.

5 CONCLUSION

Using an equation of Clapeyron-Clausius type derived in our previous study on the manifestations of the Hofmeister effect in aqueous dispersions of biomembrane lipids [1], we developed a procedure, based on differential scanning calorimetry (DSC) data, for assessing the change in the number of water molecules bound to the lipid polar headgroups at the lamellar-to-inverted hexagonal lipid phase transition. The

close proximity to published data, obtained by a different method, provides grounds to believe that the approach applied in the present work represents a promising new method for determination of the bound water amounts in lyotropic liquid crystalline phases.

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