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Thermodynamic properties of acyl chain and mesophase transitions for phospholipids in KSCN

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The presence of a one molar solution containing KSCN was shown to induce an interdigitated gel phase in dipalmitoylphosphatidylcholine which can be deduced by examining the lipid thermodynamic phase transition parameters. Specifically, the sub- and pre-transitions can no longer be observed even by low resolution calorimetry. The main transition was completely reversible, however, with scan repetition. We concluded that the presence of 1 M KSCN induces an interdigitated bilayer phase in saturated chain phosphatidylcholines but not saturated chain phosphatidylethanolamines. The lamellar to non-lamellar phase transition was also examined in dielaideoylphosphatidylethanolamine. The presence of 1 M KSCN raised the L_α to H_{II} phase transition temperature indicating that the lamellar phase has been stabilized. The presence of KSCN thus affects the packing within the bilayer such that it is the 'preferred' thermodynamic phase.

Introduction

Biological membranes consist of a milieu of lipids and proteins which determine their structure and function. The ability of some lipids to form non-lamellar structures has been implicated in a variety of functions which involve the contact and fusion of membrane segments [1]. The physiological conditions required to induce the lamellar to non-lamellar phase transition in localized portions of the membrane must be related to physico-chemical parameters which determine the stability of this phase. In addition, a variety of lipid systems have recently been shown to form an interdigitated bilayer phase in the presence of non-aqueous solvents [2–5] and salt solutions [6–8]. In order to elucidate the conditions which determine phase stability in lipid model or biological membranes, a systematic examination of these conditions must be accomplished. One aspect of this problem is the determination of the factors influencing the stability of the lipid-solvent surface. Gruner and colleagues [9,10] have shown that the surface structure of this interface determines the radius of curvature of the lipids within a pre-disposed phase. Of course this factor is also related to the conditions which cause the formation of interdigitated bilayer phases.

In particular, molar solutions of KSCN or NaSCN have recently been shown to affect the lipid–water interaction sufficiently to induce phase changes. Cunningham and Lis [6] have shown that DPPC in 1 M KSCN forms in interdigitated bilayer phase, while Yeagle and Sen [11] have shown that in 1 M NaSCN PE's will have their lamellar phase stabilized as compared to the hexagonal II (or cylindrical) phase. In both cases, arguments have been presented to justify these phase transformations in terms of the effect of SCN^- on water structure and solutilization properties. However, it is not as yet clear that this is a general phenomenon in all lipid systems.

In this report, we have examined the effect of 1 M KSCN on a variety of phosphatidylcholines, phosphatidylethanolamines and bovine brain sphingomyelin. X-ray diffraction and scanning calorimetry were used to determine the phases present and their thermodynamic

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Abbreviations: DMPC, DPPC and DSPC, respectively, dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholine; DEPE, DLPE and DMPE, respectively, dielaideoyl-, dilauroyl- and dimyristoylphosphatidylethanolamine.

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transition properties. In general, the presence of 1 M KSCN favored the formation of interdigitated bilayer phases in PC's, and the bilayer phase in DEPE. The influence of this solvent on the saturated chain phosphatidylethanolamine derivatives, DLPE and DMPE, and bovine brain sphingomyelin was more complicated.

Materials and Methods

All phospholipids were obtained from Avanti Polar Lipids (Birmingham, AL) and bovine brain sphingomyelin was obtained from Sigma Chemical Co. (St. Louis, MO). All lipids were used without further purification. The reagent grade salt was obtained from Fisher Chemical Co. Distilled water was used throughout. Lipid dispersions were prepared by suspending the lipid in distilled water of 1 M KSCN solution at approx. 80 °C until a uniform phase was observed. All dispersions were then equilibrated at approx. 0 °C for over one week. Samples for calorimetric measurement were hermetically sealed in aluminum pans, while those for X-ray diffraction measurements were mounted between microsheets.

Transition temperatures and enthalpies were measured by a Perkin-Elmer DSC-2C. At least two separate DSC scans were obtained for each sample system. Transition temperatures are exact to within 1 C°, while enthalpies have a measured uncertainty of less than 10%. Analyses of the measured thermograms were made with a Perkin Elmer Thermal Analysis Data Station (TADS) using the as supplied software to determine the peak enthalpy and onset temperature of the transition. Enthalpy per unit area was calibrated using indum (99.999% pure). The heating rate was 2.5 min for samples and calibration.

Samples were also exposed to nickel filtered CU K_α ($\lambda = 1.540$ Å) radiation. The X-ray patterns were collected photographically using modified Guinier type cameras. Bragg diffraction was used to determine the bilayer repeat spacing (d), and powdered teflon was used as an internal standard for measuring the d spacings. A circulating water bath was used to change and maintain the sample temperature.

Results and Discussion

Samples of DPPC in water and 1 M KSCN have been previously examined and the results of the examination published. The equilibration of DPPC in 1 M KSCN results in a bilayer phase which has interdigitated chains as opposed to the usual gel bilayer phase [6–8]. The thermogram which is produced contained a single endotherm at approximately the same transition temperature as DPPC in water. Samples containing DMPC in 1 M KSCN (Table I) also produced thermograms with a single endotherm which we inferred to result from the presence of interdigitated chains in the gel phase. Although we were not successful in obtaining thermograms for DLPC in 1 M KSCN, we have used X-ray diffraction to examine the room temperature phase (Table II). At all solvent concentrations used,

TABLE I

Thermodynamic parameters for lipids dispersed in water or 1 M KSCN

All scans were obtained after the sample was allowed to equilibrate at approx. 0 °C for over 7 days. The scan rate for each experiment was 2.5 K/min.

Lipid	Solvent	T_1 (K)	ΔH_1 (kcal/mol)	T_2 (K)	ΔH_2 (kcal/mol)	T_3 (K)	ΔH_3 (kcal/mol)
DLPE	H_2O	306.0	11.3	315.4	2.40		
	1 M KSCN	310.3	6.67				
DMPE	H_2O	300.0	0.47	311.7	0.58	322.4	5.58
	1 M KSCN	311.8	3.42	316.8	5.54	326.6	3.49
DEPE	H_2O	311.0	1.93	324.0	2.47		
	1 M KSCN	304.8	6.69				
DMPC	H_2O	288.9	0.50	297.3	5.46		
	1 M KSCN			294.2	7.23		
DPPC	H_2O	294.0	2.53	308.3	0.98	314.3	7.26
	1 M KSCN	–	–	–	–	311.5	8.34
DSPC	H_2O	300.0	1.52	323.5	0.93	327.2	8.09
	1 M KSCN			326.4	2.05	329.2	8.23
Sphingo-myelin	H_2O	302.4	2.53				
	1 M KSCN	294.1	2.19				

TABLE II

Structural parameters for DLPC bilayer equilibrated in 1 M KSCN.

Wt% lipid	d spacing (Å)	d_l (Å)	d_w (Å)
74.2	41.9	31.1	10.8
64.7	47.3	30.6	16.7
60.9	47.3	28.8	18.5
56.3	48.3	27.2	21.1

DLPC bilayers in 1 M KSCN have d spacing significantly smaller than that produced when fully hydrated [15] in water ($d = 59$ Å, $d_l = 30$ Å, and $d_w = 29$ Å). It was previously shown [6] that the bilayer d spacing for gel state DPPC in 1 M KSCN increased with increasing solvent until the induction of a coexisting bilayer phase with a significantly lower d spacing. Each phase continued to swell with further increases in the solvent content until the larger bilayer phase disappeared. The second phase continued to swell with increasing solvent until full hydration occurred with the resulting stabilization of the bilayer mesophase dimension. The mesophase d spacing and acyl chain diffraction peak were used to confirm that the fully hydrated gel state DPPC bilayer in 1 M KSCN was interdigitated. The presence of a significantly smaller gel state bilayer spacing than that observed in the L_β or $L_{\beta'}$ phase, near or at full hydration is one indication of the possible formation of an interdigitated bilayer phase. There is, in addition, some evidence of an ordered packing of acyl chains as evidenced by a distinct wide-angle diffraction line. We can conclude that the gel state bilayers of DLPC, DMPC and DPPC contain interdigitated chains when in the presence of 1 M KSCN.

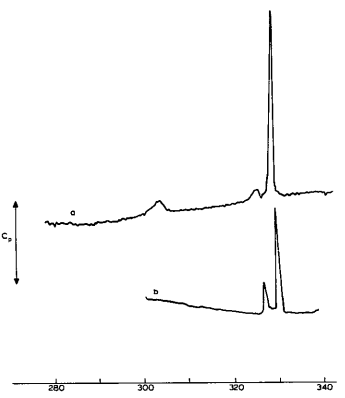

Fig. 1. Calorimetry scans (specific heat at constant pressure (kcal/K per g lipid) versus temperature) for DSPC in (a) H_2O and (b) 1 M KSCN. The specific heat scale is set so that endothermic phase transitions produce positive peaks while exothermic phase transitions produce negative peaks. An arbitrary C_p scaling is used to maximize appearance in the figure. Exact thermodynamic parameters of the phase transitions were given in Table I. The scan rate was 2.5 K/min.

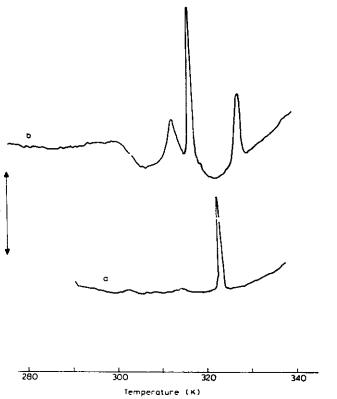

Fig. 2. Calorimetry scans (specific heat at constant pressure (kcal/K per g lipid) versus temperature) for DMPE in (a) H_2O and (b) 1 M KSCN. The specific heat scale is set so that indothermic phase transitions produce positive peaks while exothermic phase transitions produce negative peaks. An arbitrary C_p scaling is used to maximize appearance in the figure. Exact thermodynamic parameters of the phase transitions were given in Table I. The scan rate was 2.5 K/min.

Samples of DSPC in 1 M KSCN, however, produced two distinct endotherms at temperatures approximately (slightly higher) those of the pre- and main-phase transition for DSPC in water (Fig. 1). It is unlikely that the low-temperature phase in this system contains interdigitated acyl chains. We can infer that the phase sequence for the observed transitions is: $L_c \rightarrow L_\beta$ (interdigitated) $\rightarrow L_\alpha$. Of course, we cannot rule out that the thermograms are somehow related to K^+ binding to DSPC headgroups.

Samples of DLPE and DMPE were also examined in water and 1 M KSCN (Table I). DLPE in water equilibrated as described in Materials and Methods produced two endotherms during the initial heating cycle which can be assigned to the transitions between a subgel (L_c) and liquid crystal (L_α) phase. A single

endotherm was observed for DLPE in 1 M KSCN which is similar in character to that of the $L_{\beta} \rightarrow L_{\alpha}$ transition observed in water. Samples of DMPE produced sub-, pre- and main transitions during initial heating in both water and 1 M KSCN (Fig. 2). The pre- and subtransitions have higher enthalpies and the main transition a lower enthalpy when 1 M KSCN was present instead of water. There is no thermodynamic evidence that chain interdigitation occurred in either of the PE's in the presence of 1 M KSCN.

Samples of bovine brain sphingomyelin were also studied in water and 1 M KSCN. From the differences in transition temperature and enthalpy in these systems it can be inferred that sphingomyelin was in a less ordered state when 1 M KSCN was present in place of water. There is no evidence for the presence of an interdigitated phase.

Finally, a previous report had indicated that the presence of 1 M NaSCN raised the transition temperature of the $L_{\alpha} \rightarrow H_{II}$ transition in naturally derived phosphatidylethanolamine. Thermodynamic analysis of the transitions for DEPE in water and 1 M KSCN is consistent with this observation. We observed no evidence of the $L_{\alpha} \rightarrow H_{II}$ transition up to 360 K for DEPE in the presence of 1 M KSCN, although this transition was observed by Epand and Bryszewska [16] to occur at 360 K in the presence of NaSCN. This transition occurs at 324 K for DEPE in water (Table I).

Conclusion

It is apparent that the presence of 1 M KSCN causes interdigitated bilayers in phosphatidylcholines but not phosphatidylethanolamines, and raises the transition temperature of the $L_{\alpha} \rightarrow H_{II}$ transition in phosphatidylethanolamines. We must probe the relationship between the solvent structure and the lipid interface in order to justify these observations. Clearly, ions in solution are not solely responsible for these observations since fused salt, (ethylammonium nitrate), causes PC and PE molecules to favor the formation of H_{II} phases at elevated temperatures [12–14]. Furthermore, we have determined that the forces between bilayers are affected by various monovalent salt solutions but that comparison of different ionic solutions indicates that there are no quantitative differences in these forces.

It is known that SCN^- is a chaotropic ion which tends to disrupt the structure of water. It has been argued that this disruption would reduce the hydrogen bonding network, loosen the packing between lipid molecules and stabilize the bilayer phase. This argument would explain the results observed for PE's and 1 M KSCN. Less obvious is the relationship of water structure and PC mesophases. Hydrogen bonding and water structure is clearly a factor in all bilayer formation. If the hydrogen bonding network around a gel state PC bilayer were loosened, there could be a sufficient increase in the area per lipid headgroup to allow molecular rotations which could form some of the proposed interdigitated gel bilayer structures. However, these structures would not be those with necessarily the closest packed headgroup structures. In general, though, it is evident that whatever change SCN^- makes in the solvent structures appears to stabilize the bilayer phase.

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