Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Modulation of Phospholipid Phase Structures and Transitions Induced by Oxysterols

L J. Lis [a], O. Kucuk [a], M. P. Westerman [a], W. Tamura-Lis [b], P. J. Quinn [c], B. A. Cunningham [d], J. M. Collins [e] & D. H. Wolfe [f]

[a] Division of Hematology/Oncology, The Chicago Medical School, Mount Sinai Hospital Medical Center and The Veterans Affairs Medical Center, N. Chicago, IL, 60064

[b] University of Nebraska Medical Center, Omaha, NE, 68105

[c] Department of Biochemistry, King's College, Campden Hill Road, London, W8 7AH, U.K.

[d] Department of Physics, Bucknell University, Lewisburg, PA, 17837

[e] Department of Physics, Marquette University, Milwaukee, W153233

[f] Department of Astronomy and Physics, Lycoming College, Williamsport, PA, 17701

Version of record first published: 24 Sep 2006.

Mol. Cryst. Liq. Cryst., 1992, Vol. 213, pp. 213–222
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United States of America

Modulation of Phospholipid Phase Structures and Transitions Induced by Oxysterols

L. J. LIS,† O. KUCUK,† M. P. WESTERMAN,† W. TAMURA-LIS,‡ P. J. QUINN,§ B. A. CUNNINGHAM,‖ J. M. COLLINS¶ and D. H. WOLFE**

†*Division of Hematology/Oncology, The Chicago Medical School, Mount Sinai Hospital Medical Center and The Veterans Affairs Medical Center, N. Chicago, IL 60064;* ‡*University of Nebraska Medical Center, Omaha, NE 68105;* §*Department of Biochemistry, King's College, London, Campden Hill Road, London W8 7AH, U.K.;* ‖*Department of Physics, Bucknell University, Lewisburg, PA 17837;* ¶*Department of Physics, Marquette University, Milwaukee, WI 53233 and* ***Department of Astronomy and Physics, Lycoming College, Williamsport, PA 17701*

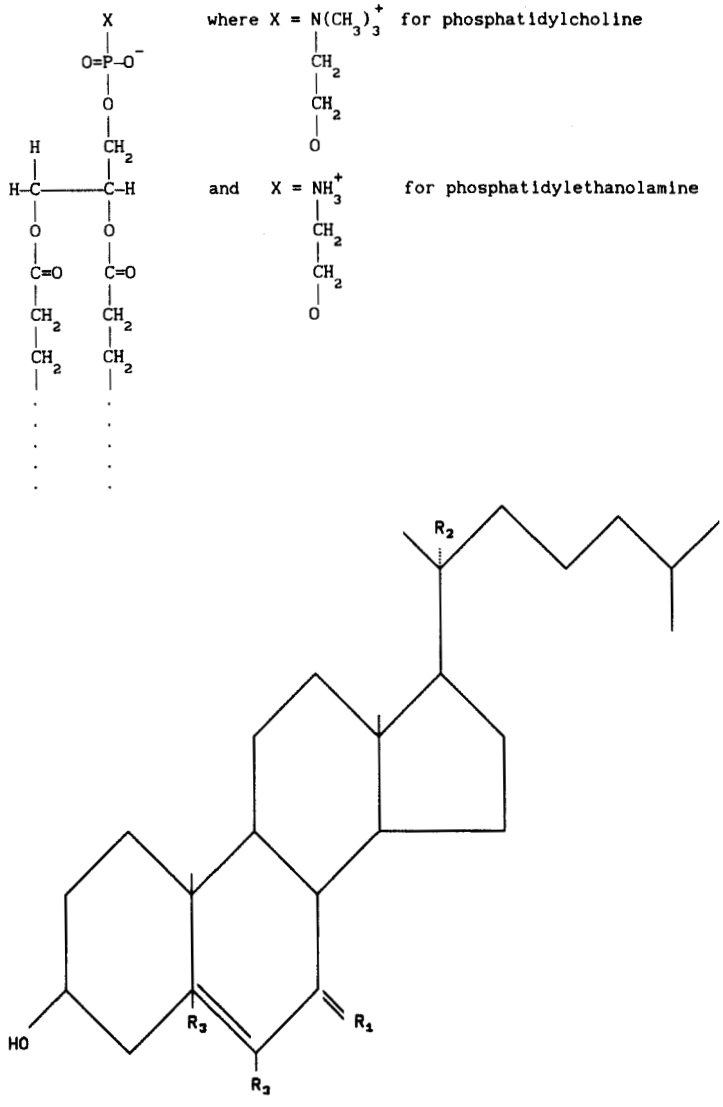
(Received January 30, 1991; in final form October 8, 1991)

Admixtures of various oxidized sterol compounds and 1-palmitoyl, 2-oleoyl phosphatidylethanolamine (POPE) in buffer, or 1,2 *O*-dihexadecylphosphatidylcholine (DHPC) in water were examined using real time x-ray diffraction. The formation of non-lamellar POPE phases was enhanced by the presence of 5 mole% cholesterol, 7-ketocholesterol, or 20α-hydroxycholesterol, but not by the presence of 5 mole% 5α-cholestane-3β,5,6β-triol. The presence of 5 mole% cholesterol, 7-ketocholesterol or 5α-cholestane-3β,5,6β-triol was sufficient to eliminate the DHPC interdigitated gel bilayer phase over its usual temperature range and to replace it with a gel state ripple phase. Sterol-phospholipid interactions thus resulted in a direct conversion from the sub gel bilayer phase to the gel state ripple phase for DHPC mixtures with 5 mole% cholesterol, 7-ketocholesterol, or 5α-cholestane-3β,5,6β-triol rather than the sequence of interdigitated sub gel bilayer phase to interdigitated gel state bilayer phase to a final state of gel state ripple phase observed for DHPC in water. These results can be used to interpret the extent of sterol influence on the intra-bilayer interactions between lipid molecules.

INTRODUCTION

Cholesterol is a major component of both biological membranes and liposomal preparations. Much is known regarding how cholesterol influences phase transition parameters but not how it directly interacts with the lipid molecules in bilayers. One of the protocols developed to attempt to understand cholesterol-phospholipid interactions is to use sterols that contain slight differences in moieties attached to the sterol ring and/or phospholipids with different headgroup moieties (Figure 1). If oxidized sterols are used as cholesterol derivatives, one can also learn how phospholipid-cholesterol interactions are changed by the oxidation of specific sites on the cholesterol molecule.

Lipid model membranes have been extensively characterized for their lyotropic and thermotropic liquid crystalline phase behavior for over thirty years (for typical



where R_1= O and no double bond between R_3's for 7-ketocholesterol
R_2= OH for 20 α-hydroxycholesterol
and R_3= OH for 5α-cholestane-3β,5,6β-triol

FIGURE 1 Lipid and sterol structures.

phase structures see Figure 2). These studies have been helpful in directly establishing the physical and chemical conditions for lipid phase transformation into rather unique lamellar and non-lamellar phases and directly allowing an inference as to how lipid molecules interact within a phase. Theoretical models[1–6] have been described which help to predict non-lamellar phase formation based on the structure

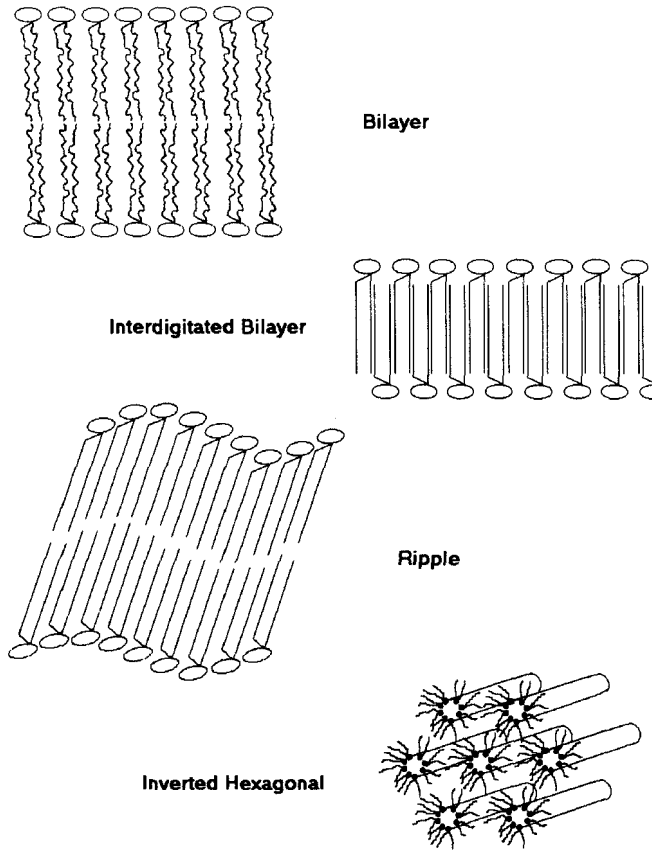


FIGURE 2 Lipid phases. Note: bilayers, ripple phases, and interdigitated phases can contain subgel (crystalline), gel, or liquid crystalline (disordered) acyl chains while the inverted hexagonal phase only contains disordered acyl chains.

of the lipid-solvent interface and changes in acyl chain packing. There have also been a number of theoretical approaches to describing how and/or why ripple phases form.[7–10] Recently, Cevc[11] has provided a unified theory to explain both ripple and interdigitated phase formation. The driving force for ripple phase formation was deduced to be the interfacial tendency for a lateral expansion (the headgroup-water-headgroup repulsion). It was predicted that the smaller the interfacial contribution the closer the pre-transition (gel state bilayer to ripple phase transition) temperature shifts to the main transition (acyl chain melting transition) temperature while larger contributions cause the pre-transition temperature to shift toward the sub-transition (crystalline to gel state acyl chain transition) temperature. Additionally, the wave-vector of the ripple phase was defined in terms of the hydration state of the lipid. An extreme case combining both hydration and acyl chain interaction components was described as the interdigitated phase. The sub-transition and main transition were seen as being primarily driven by acyl chain transformations.

In this report, we correlate changes in the ability of 1-palmitoyl, 2-oleoyl phos-

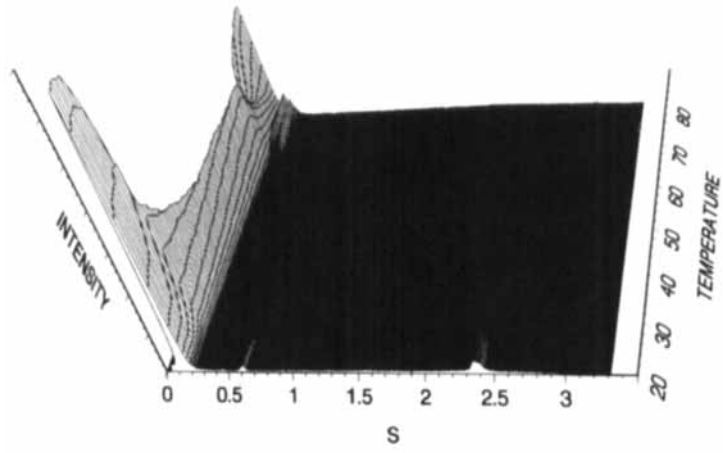


FIGURE 3 Three dimensional plot of scattering intensity versus reciprocal spacing (S) for POPE in TES undergoing a heating scan at a rate of 5°/min. Every third diffraction pattern of 3s duration in the data set is shown. Temperature units are in °C and reciprocal space units are in nm^{-1}, with intensity in arbitrary units.

phatidylethanolamine (POPE) to form non-lamellar phases, and dihexadecylphosphatidylcholine (DHPC) to form ripple phases in the presence of oxidized sterols to cholesterol-phospholipid interactions. Samples containing only 5 mole% sterol were examined using real time x-ray diffraction[12] to minimize the influence of domains of pure sterol on our analyses of interactions between phospholipid headgroups.

MATERIALS AND METHODS

1-Palmitoyl,2-oleoyl phosphatidylethanolamine was obtained from Avanti Polar Lipids (Birmingham, AL), and 1,2 O-dihexadecylphosphatidylcholine was obtained from Sigma Chemical Co. (St. Louis, MO). Sterols were obtained from Sigma Chemical Co. (St. Louis, MO), and Steraloids, Inc. (Milton, NH). All water was distilled and salts were reagent grade.

Lipid mixtures were made by weighing known amounts of lipid powders which were subsequently co-solubilized in chloroform. Chloroform was removed via rotary evaporation, with a final drying under vacuum. Lipids were kept under N_2 at -20°C until mixed with water or TES buffer. DHPC samples were equilibrated at ~60°C until no unhydrated powder was observed while POPE samples were subjected to at least three freeze/thaw cycles. DHPC samples for x-ray examination were cooled to at least -50°C and then raised to the desired initial temperature in the x-ray sample holders before x-ray examination.

X-ray experiments were performed using a monochromatic (0.15 nm) focused x-ray beam at station 8.2 of the Daresbury (U.K.) Synchrotron Laboratory. A purpose built camera allowed clear resolution of reflections from 10 nm down to 0.35 nm. The sample holder was a cryo-stage (Linkam) to which mica windows

were fitted. The sample size was about 30 μl with a path length of 1 mm. The stage was cooled to liquid nitrogen temperature and heated to the required temperature by embedded heating elements in the stage. Temperature programming over the range $-50°$ to 200°C at rates between 0.001°/min to 2.3°/sec was possible. Sample temperature was monitored by a thermocouple embedded near the sample.

X-ray data was collected on a single wire linear detector fabricated at the Daresbury Lab. The acquired data was stored in a VAX-11/750 computer and corrected for detector response by comparison with a pattern recorded using a fixed source and averaged over several hours. Data was analyzed using the OTOKO program provided by the Daresbury Laboratory. Phase transition temperatures were taken as the temperature in which the recorded diffraction peaks started to shift in *d*-spacing or additional peaks from the subsequent phase appeared.

RESULTS

The effects of cholesterol and a variety of oxysterols on the disordered bilayer (L_α) to inverted hexagonal (H_{II}) state phase transition in POPE has been examined using real time x-ray diffraction techniques. Figure 3 shows an array of diffraction

TABLE I

Structural parameters and transition temperatures for the $L_\alpha \rightarrow H_{II}$ phase transition for fully hydrated POPE admixtures containing 5 mole% sterols

Sterol	Phase	Mesophase d-spacings (nm)	Transition Temperature (°C)
None	L_α	5.37	69.6
	H_{II}	5.80	
cholesterol	L_α	5.52	65.3
	H_{II}	5.52	
5α-cholestane-3β,5,6β-triol	L_α	5.36	70.4
	H_{II}	5.54	
7-ketocholesterol	L_α	5.04	62.8
	H_{II}	5.44	
20α-hydroxycholesterol	L_α	5.36	61.8
	H_{II}	5.71	

Note: d-spacings for the L_α phase continuously decrease while undergoing the $L_\alpha \rightarrow H_{II}$ phase transition.

patterns collected while a sample of POPE was undergoing this phase transformation. These data indicate that POPE in TES undergoes the $L_\alpha \rightarrow H_{II}$ phase transition at approximately 70°C as determined by a change in the small angle scattering. This is consistent with previous calorimetric determinations of the temperature for this transition.[13,14] Specifically, an L_α multibilayer array with a repeat spacing of approximately 5.4 nm transforms into an hexagonal array of water cylinders embedded in a lipid phase with a repeat spacing of approximately 5.8 nm via the co-existence of the initial and final states. This transition involving incommensurate structures requires over 10° to achieve completion. As shown in Table I, the presence of approximately 5 mole% cholesterol, 7-ketocholesterol or 20α-hydroxycholesterol lowered the $L_\alpha \rightarrow H_{II}$ transition temperature (T_h) while the presence of approximately 5 mole% 5α-cholestane-3β,5,6β-triol stabilized the L_α phase at the expense of the H_{II} phase. In most cases, the addition of sterols caused a decrease in the repeat spacings of all the phases present which would indicate a probable decrease in the water separation between the lipid interfaces. This is consistent with an increase in the lipid interfacial interactions induced by the presence of sterols.

The effect of cholesterol, 7-ketocholesterol, and 5α-cholestane-3β,5,6β-triol on the DHPC phase structures and transitions was also examined using time resolved x-ray diffraction. It has been previously shown[15] that fully hydrated DHPC can form interdigitated gel state bilayers (L_β(inter)), ripple (P_β) phases, and disordered

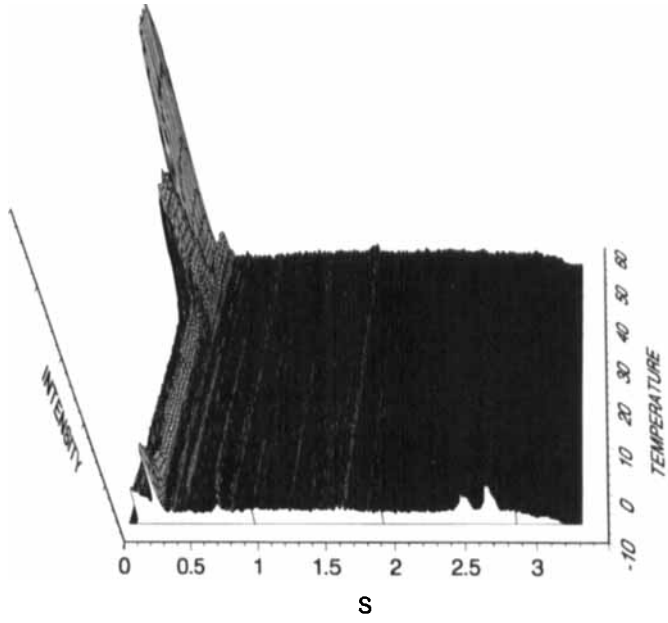


FIGURE 4 Three dimensional plot of scattering intensity versus reciprocal spacing (S) for DHPC in water undergoing a heating scan at a rate of 5°/min. Every third diffraction pattern of 3s duration in the data set is shown. Temperature units are in °C and reciprocal space units are in nm^{-1}, with intensity in arbitrary units.

bilayers (L_α) as a function of temperature. Fully hydrated DHPC was found to undergo, over the temperature studied, the transition sequence (Figure 4):

$$L_c(\text{inter}) \rightarrow L_\beta(\text{inter}) \rightarrow P_\beta.$$

The presence of interdigitated bilayer phases in the subgel phase (L_c(inter)) was inferred from the size of the bilayer spacing and the acyl chain packing.[16] Specifically, an L_c(inter) multibilayer array with a repeat spacing of approximately 4.7 nm and acyl chain scattering peaks at 0.41 and 0.39 nm transforms via a process involving the gradual elimination of the 0.39 nm scattering peak into an L_β(inter) multilamellar array with a repeat spacing of approx. 4.8 nm at approx. 5.6°C. It would be expected that the small *d*-spacing of the L_c mesophase is indicative of the presence of interdigitated chains since it is similar to that of the interdigitated gel state bilayer and that it is unlikely that an interdigitated gel state bilayer would transform to a "more disordered" non-interdigitated subgel phase. Further evidence can be obtained by comparison to the non-interdigitated phase of DPPC which is an analogue of DHPC. Note that in the case of DPPC, the *d*-spacing of the L_c phase was previously determined to be *ca* 6.0 nm[16] which is approximately

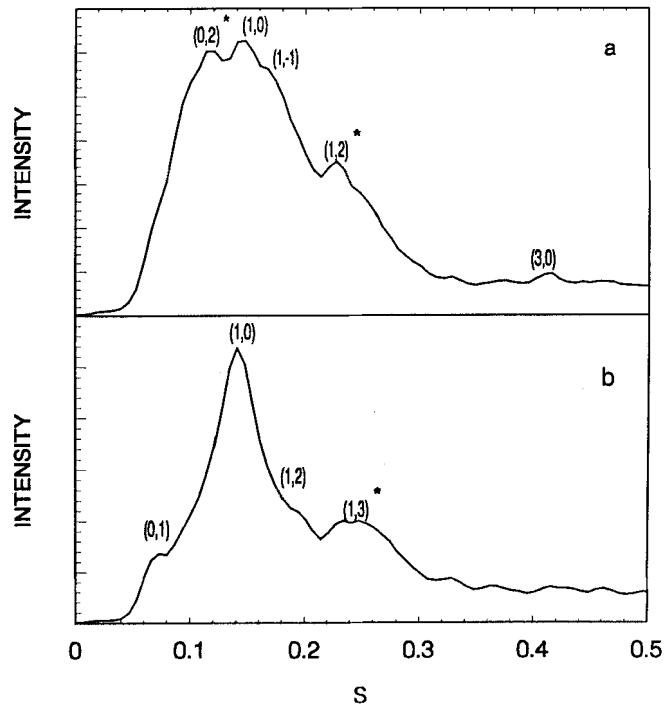


FIGURE 5 Characteristic x-ray patterns for the gel state ripple phase in a) DHPC at 41°C and b) DHPC + 5 mole% 5α-cholestane-3β,5,6β-triol at 5°C. The Miller indices of the peak assignments are indicated in parentheses. Reciprocal space units are in nm^{-1} and intensity is in arbitrary units. The assignments of Miller indices denoted by * are not conclusive.

the same *d*-spacing as the gel phase bilayers. At approx. 35.5°C, the DHPC gel state interdigitated bilayer phase transforms via a two state process, involving the co-existence of the initial and final two dimensional mesophase structures, into a P_β state with a bilayer repeat spacing of 7.15 nm and a much larger ripple repeat. The presence of the P_β phase is indicated by the superposition of two independent x-ray patterns representing the repeat spacing of the multibilayer array, and the longer ranged repeat of the ripple structure (Figure 5a). The exact determination of the ripple repeat spacing was hampered by the spatial resolution limitations in this experiment. The *d*-spacings deduced for the DHPC phases from the dynamic x-ray diffraction patterns is consistent with those previously reported[15] for temperature equilibrated samples. The presence of ~5 mole% cholesterol, 7-ketocholesterol, or 5α-cholestane-3β,5,6β-triol resulted in DHPC undergoing the temperature induced phase sequence: $L_c \rightarrow P_\beta$, with no apparent indication of the formation of an intermediate L_β phase (see Figure 6 and Table II). The presence of the non-interdigitated L_c phases in these systems is inferred by the presence of mesophases with larger *d*-spacings than for pure DHPC in water (Table II). The presence of a P_β phase in these systems is inferred from the presence of two superimposed x-ray patterns which can be independently indexed to two different unit cells; a bilayer and the larger ripple (see Figure 5b). We cannot unambiguously rule out the presence of two multibilayer phases which do not involve rippling, although the *d*-spacing of at least one of each set of phases is clearly larger than that expected for a non-charged lipid phase. The elimination of the DHPC L_β phase due to the introduction of the various sterol compounds is a further indication that sterols increase the lipid headgroup interactions.

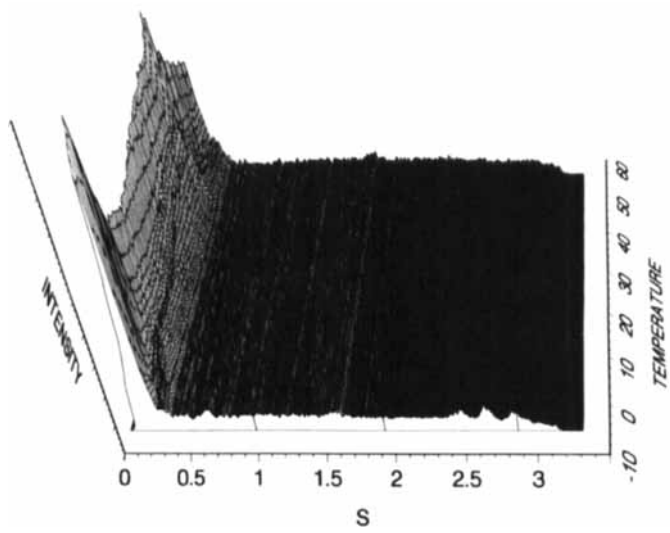


FIGURE 6 Three dimensional plot of scattering intensity versus reciprocal spacing (S) for DHPC + 5 mole% 5α-cholestane-3β,5,6β-triol in water undergoing a heating scan at a rate of 5°/min. Every third diffraction pattern of 3s duration in the data set is shown. Temperature units are in °C and reciprocal space units are in nm^{-1}, with intensity in arbitrary units.

TABLE II

Structural parameters and transition temperatures for phases produced by fully hydrated DHPC admixtures containing 5 mole% sterols

Sterol	Phase	Mesophase d-spacings (nm)	acyl chain scattering peaks (nm)	Transition Temperatures (°C)
None	$L_c(I)$	4.65	0.41, 0.39	5.6
	$L_\beta(I)$	4.75	0.41	35.5
	P_β	7.15 (> 10.0 nm)	0.42	
cholesterol	L_c	6.17	0.41, 0.39, 0.36	0.7
	P_β	7.54 (> 10.0 nm)	0.41	
5α-cholestane-3β,5,6β-triol	L_c	5.48	0.41, 0.39, 0.36	-0.5
	P_β	6.58 (> 10.0 nm)	0.41	
7-ketocholesterol	L_c	5.67	0.41, 0.38, 0.30	0.9
	P_β	6.84 (> 10.0 nm)	0.41	

Note: (1) Lamellar d-spacing increasing during phase transformations.
(2) Ripple d-spacings, in parenthesis, decrease during phase transformations.

DISCUSSION

There are a number of conclusions that can be drawn regarding the influence of sterols on the $L_\alpha \rightarrow H_{II}$ phase transitions in phosphatidylethanolamines. The change in the transition temperature coupled with the changes in the H_{II} repeat spacing can be used to infer that the presence of cholesterol, 7-ketocholesterol, and 20α-hydroxycholesterol enhances the formation of H_{II} phases for POPE while the presence of 5α-cholestane-3β,5,6β-triol does not. It can be concluded that oxysterols oxidized at multiple sites may have a greater influence on the stability of the bilayer phase, while less oxidized oxysterols favor the formation of non-lamellar lipid phases. A number of general conclusions can be formed on the possible influence of oxysterols on lipid morphology from our studies with DHPC. The presence of oxysterols tends to stabilize the thermomechanical fluctuation responsible for the ripple phase formation in DHPC bilayers due to a generalized sterol induced increase in the intra-membrane interfacial interactions. This is opposite to what is observed when the solvent structure adjacent to DHPC bilayers is altered by the addition of alcohols.[17] The presence of oxysterols thus reduces the ability of the lipid bilayer to dissipate the mechanical stresses formed as the lipid headgroup

areas enlarge due to increasing temperature. The lack of a molecular mechanism for stress dissipation results in a need for the membrane itself to be deformed. The sterol induced elimination of the interdigitated subgel phase is indicative of modifications (i.e., a lessening) of the acyl chain interactions.

Finally, these data allow one to compare the headgroup contributions to phase transitions involving non-lamellar phases. The transformation to the ripple phase is driven by headgroup interactions while that resulting in the interdigitated phase is driven by acyl chain interactions. Our data indicate that sterols influence both types of interactions in DHPC suggesting that sterols have the ability to modify both types of interactions stabilizing the formation of ripple phases. It can be concluded that the mode of interaction for cholesterol, 7-ketocholesterol, and 20α-hydroxycholesterol in stabilizing the POPE H_{II} phase is between headgroups while that for 5α-cholestane-3β,5,6β-triol in stabilizing the POPE L_α phase is acyl chain in origin. This information may be important in efforts to define the position and conformation of various sterols within lipid bilayers.

Acknowledgments

This work was supported in part by Mount Sinai Hospital Service Club and by the VA Merit Review Board. We thank Dr. Wim Bras of the Daresbury Synchrotron Laboratory for his technical assistance and scientific advice.

References

1. D. Siegel, *Biophys. J.*, **49**, 1155–1170 (1986).
2. D. Siegel, *Biophys. J.*, **49**, 1171–1183 (1986).
3. D. Siegel, *Chem. Phys. Lipids*, **42**, 279–301 (1986).
4. G. L. Kirk, S. M. Gruner and D. L. Stein, *Biochemistry*, **23**, 1093–1102 (1984).
5. S. M. Gruner, *Proc. Natl. Acad. Sci. USA*, **82**, 3665–3669 (1985).
6. S. M. Gruner, M. W. Tate, G. L. Kirk, P. T. C. So, D. C. Turner, D. T. Keane, G. P. S. Tilcock and P. R. Cullis, *Biochemistry*, **27**, 2853–2866 (1988).
7. M. Marclu, H. L. Frisch, J. S. Langer and H. M. McConnell, *Proc. Natl. Acad. Sci. USA*, **81**, 6559–6560 (1984).
8. R. E. Goldstein and S. Leibler, *Phys. Rev. Lett.*, **61**, 2213–2216 (1988).
9. W. S. McCullough and H. L. Scott, *Phys. Rev. Lett.*, **65**, 931–934 (1990).
10. W. S. McCullough, J. H. H. Perk and H. L. Scott, *J. Chem. Phys.*, **93**, 6070–6080 (1990).
11. G. Cevc, *Biochim. Biophys. Acta*, **1062**, 59–69 (1991).
12. L. J. Lis and P. J. Quinn, *J. Appl. Cryst.*, **24**, 48–60 (1991).
13. R. M. Epand and R. Bottega, *Biochim. Biophys. Acta*, **944**, 144–154 (1988).
14. D. P. Siegel, J. Banschback, D. Alford, H. Ellens, L. J. Lis, P. J. Quinn, P. L. Yeagle and J. Bentz, *Biochemistry*, **28**, 3703–3709 (1989).
15. P. Laggner, K. Lohner, G. Degovics, K. Miller and A. Schuster, *Chem. Phys. Lipids*, **44**, 31–60 (1987).
16. B. Tenchov, L. J. Lis and P. J. Quinn, *Biochim. Biophys. Acta*, **897**, 143 –151 (1987).
17. J. A. Veiro, P. Nambi and E. S. Rowe, *Biochim. Biophys. Acta*, **943**, 108–111 (1988).