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THE PREFERENCE OF CHOLESTEROL FOR PHOSPHATIDYLCHOLINE IN MIXED PHOSPHATIDYLCHOLINE-PHOSPHATIDYLETHANOLAMINE BILAYERS

P. W. M. VAN DIJCK, B. DE KRUIJFF, L. L. M. VAN DEENEN, J. DE GIER and R. A. DEMEL

Laboratory of Biochemistry, State University of Utrecht, University Centre "De Uithof", Padualaan 8, Transitorium 3, Utrecht (The Netherlands)

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SUMMARY

The following phosphatidylethanolamines were studied by differential scanning calorimetry: 1,2-dipalmitoleoyl-, 1,2-dioleoyl-, 1,2-dilauroyl-, 1,2-dielaidyl-, 1,2-dimyristoyl- and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoryl-ethanolamine.

The saturated and trans-unsaturated species underwent thermotropic phase transitions at temperatures about 20–30 °C higher than the corresponding phosphatidylcholines but the enthalpy changes were nearly identical. The transition temperatures for the cis-unsaturated species were about the same as those of the corresponding phosphatidylcholines but here the enthalpy change was markedly decreased as compared with the phosphatidylcholines. Freeze-fracture electron microscopy revealed phase changes from a lamellar to a hexagonal phase for 1,2-dipalmitoleoyl- and 1,2-dioleoyl-*sn*-glycero-phosphorylethanolamine at 20 and 0 °C respectively. At these temperatures no transitions were apparent in the calorimeter scan.

Incorporation of increasing amounts of cholesterol into phosphatidylethanolamine bilayers gradually decreased the enthalpy changes of the phase transition in the same manner as was demonstrated before for phosphatidylcholine/cholesterol mixtures. This was studied both for 1,2-dipalmitoleoyl- and 1,2-dimyristoyl-*sn*-glycero-phosphorylethanolamine.

In an equimolar mixture of 1,2-dioleoyl- and 1,2-dipalmitoylphosphoryl-ethanolamine, which showed phase separation, cholesterol preferentially decreased the transition of the lowest melting component.

In equimolar mixtures of phosphatidylethanolamines and phosphatidylcholines, which showed phase separation, cholesterol preferentially abolished the transition of the phosphatidylcholine component present. This occurred both in experiments where the phosphatidylcholine was the lowest melting and where it was the highest melting component present in the mixture. These experiments strongly suggest that in phosphatidylcholine-phosphatidylethanolamine mixtures at temperatures where both components are in the liquid-crystalline state cholesterol is preferently associated with the phosphatidylcholine component in the mixture.

INTRODUCTION

Aqueous dispersions of lamellar lipid bilayers (liposomes) provide simplified models for the investigation of biochemical and biophysical properties of membrane lipids, yielding information which can be extrapolated to the understanding of the architecture and function of complex biomembranes. Using such models, it was shown that cholesterol in phosphatidylcholine bilayers gradually abolishes the cooperative gel to liquid-crystalline phase transition, with the magnitude of the effect being proportional to the amount of cholesterol added [1–3]. Differential scanning calorimetry measurements showed that the cooperative gel to liquid crystalline phase transition was no longer apparent at a molar ratio cholesterol to phospholipid of 1 : 2 [2, 3]. In mixtures of synthetic phosphatidylcholines which show cocrystallization it could be demonstrated that cholesterol did not show a preference for one of the components present. The transition was abolished in the same way as in single phosphatidylcholine systems. In mixtures of phosphatidylcholines which showed phase separation cholesterol preferentially interacted with the lower melting component and was frozen out of the gel phase patches where the highest melting component is concentrated. In these cases cholesterol is heterogeneously distributed in the plane of the membrane at temperatures where phase separation occurs. These calorimetric experiments [4, 5] were recently confirmed by fluorescence studies [6].

Many biological membranes contain in addition to cholesterol a variety of phospholipids with different polar head groups. To understand the lateral distribution of cholesterol in membranes the effect of cholesterol upon the thermotropic properties of bilayers composed at different phospholipids has to be studied systematically.

In this study therefore the effect of cholesterol in bilayers composed of synthetic phosphatidylcholines and phosphatidylethanolamines was investigated by differential scanning calorimetry.

MATERIALS AND METHODS

The following synthetic phosphatidylcholines were synthesized as described before [7]: 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphorylcholine (16 : 1_c/16 : 1_c-phosphatidylcholine), 1,2-dioleoyl-*sn*-glycero-3-phosphorylcholine (18 : 1_c/18 : 1_c-phosphatidylcholine), 1,2-dilauroyl-*sn*-glycero-3-phosphorylcholine (12 : 0/12 : 0-phosphatidylcholine), 1,2-dielaidoyl-*sn*-glycero-3-phosphorylcholine (18 : 1_{tr}/18 : 1_{tr}-phosphatidylcholine) 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (14 : 0/14 : 0-phosphatidylcholine) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (16 : 0/16 : 0-phosphatidylcholine).

The synthetic phosphatidylethanolamines: 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphorylethanolamine (16 : 1_c/16 : 1_c-phosphatidylethanolamine), 1,2-dioleoyl-*sn*-glycero-3-phosphorylethanolamine (18 : 1_c/18 : 1_c-phosphatidylethanolamine),1,2-dielaidoyl-*sn*-3-phosphorylethanolamine (18 : 1_{tr}/18 : 1_{tr}-phosphatidylethanolamine), 1,2-dilauroyl-*sn*-glycero-3-phosphorylethanolamine (12 : 0/12 : 0-phosphatidyl-ethanolamine) and 1,2-dimyristoyl-*sn*-glycero-3-phosphorylethanolamine (14 : 0/14 : 0-phosphatidylethanolamine) were synthesized as described by Cullis and de Kruijff [8]. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylethanolamine (16 : 0/16 : 0-phosphatidylethanolamine) was obtained by hydrogenation of 16 : 1_c/16 : 1_c-phosphatidyl-ethanolamine.

Differential scanning calorimetry experiments were performed as described

before [5], with the following modifications in the sample preparation. The phospholipids and cholesterol were mixed in chloroform and then concentrated under a stream of nitrogen. The concentrated solution was transferred to a sample pan. Chloroform was totally removed by a nitrogen stream followed by overnight high vacuum storage. The samples were hydrated by adding about 15 μl of a 40 mm Tris-acetate/ethyleneglycol (1 : 1, v/v), buffer, pH 7.0, containing 100 mm NaCl. A control experiment demonstrated no effects of ethyleneglycol on the transition temperature and energy content of the transition of 14 : 0/14 : 0-phosphatidylethanolamine. The sample pan was sealed and equilibrated above the transition temperature. Both heating and cooling scans were taken at 5 °C/min scanning speed. No equilibration times were taken between a heating and cooling scan and vice versa. Energy contents (ΔH) were calculated by relating the peak area to known calibration areas. The amount of lipid sealed in a pan was determined as follows. Sample pans were opened with forceps and sonicated in a chloroform/methanol solution in a bath sonicator until pan and cover were completely separated. Aliquots were taken from this chloroform/methanol solution and a phosphorus determination was done according to Fiske-Subbarow after perchloric acid destruction as described by Bartlett [9].

In heating curves of mixtures sometimes exo- and/or endothermic transitions of unknown origin were present. In pure phosphatidylethanolamine dispersions these transitions were not observed. Possibly they reflect a metastabile phase behaviour. Metastable transitions have also been reported for synthetic phosphatidylglycerols [10, 11]. When, in mixtures which show phase separation, these kinds of transitions occurred in the region of the transition of the lower melting component they could be eliminated by undercooling but when they occurred near the transition temperature of the upper melting component this was not possible. In cooling curves these phenomena did not occur. Therefore the cooling curves were taken for the calculation of ΔH values. Since heating curves give a more accurate determination of the transition temperatures, heating curves will be presented when necessary. In these curves the anomalous transitions were absent.

The lipid phase transition temperature (T_c) is defined as the temperature where the slope of the descending arm of the main peak intersects the baseline.

The error in the determination of the ΔH of single components and mixtures was estimated as 5 and 10 % respectively. The error in the determination of the transition temperature (T_c) was estimated as 0.5 °C. Freeze-fracturing electron microscopy was done as described before [12].

RESULTS

Since the knowledge of the thermotropic behaviour of synthetic phosphatidylethanolamines is still very limited we first compared the transition temperatures and the enthalpy changes involved in the transitions of the synthetic phosphatidylcholines with the corresponding synthetic phosphatidylethanolamines (Fig. 1). In agreement with other reports [13–16], saturated phosphatidylethanolamines (12 : 0/12 : 0-, 14 : 0/14 : 0-, and 16 : 0/16 : 0-phosphatidylethanolamines) undergo a lipid phase transition some 20–30 °C higher than the corresponding phosphatidylcholines and

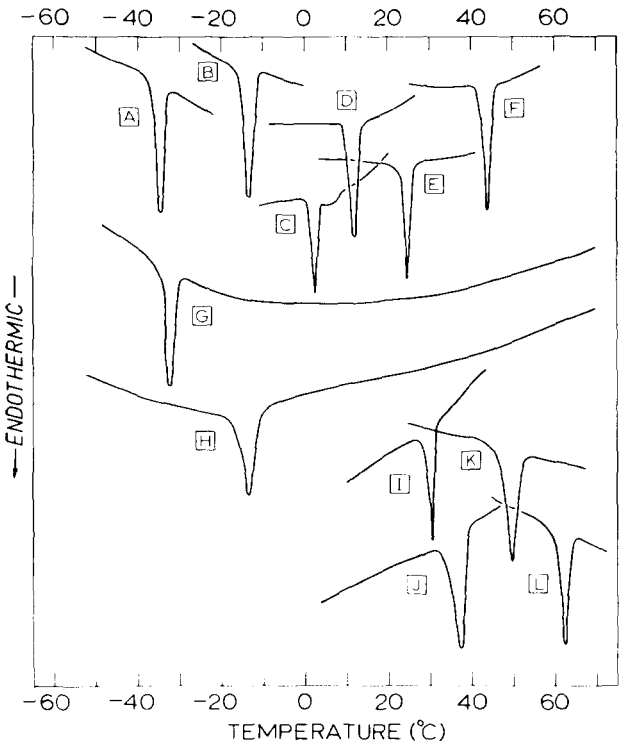

Fig. 1. A comparison between the thermotropic properties of some synthetic phosphatidylcholines and phosphatidylethanolamines. Heating scans were made as described in Materials and Methods. (A) 16 : 1_c/16 : 1_c-phosphatidylcholine: T_c — 36 °C, ΔH 9.1 kcal/mol; (B) 18 : 1_c/18 : 1_c-phosphatidylcholine: T_c —14 °C, ΔH 11.2 kcal/mol; (C) 12 : 0/12 : 0-phosphatidylcholine: T_c 0 °C, ΔH 4.3 kcal/mol; (D) 18 : 1_{tr}/18 : 1_{tr}-phosphatidylcholine: T_c 9.5 °C, ΔH 7.3 kcal/mol; (E) 14 : 0/14 : 0-phosphatidylcholine: T_c 23 °C, ΔH 6.8 kcal/mol; (F) 16 : 0/16 : 0-phosphatidylcholine: T_c 41.5 °C, ΔH 8.6 kcal/mol; (G) 16 : 1_c/16 : 1_c-phosphatidylethanolamine: T_c —33.5 °C, ΔH 4.3 kcal/mol; (H) 18 : 1_c/18 : 1_c-phosphatidylethanolamine: T_c —16 °C, ΔH 4.5 kcal/mol; (I) 12 : 0/12 : 0-phosphatidylethanolamine: T_c 29 °C, ΔH 4.0 kcal/mol; (J) 18 : 1_{tr}/18 : 1_{tr}-phosphatidylethanolamine: T_c 35 °C, ΔH 7.0 kcal/mol; (K) 14 : 0/14 : 0-phosphatidylethanolamine: T_c 47.5 °C, ΔH 6.4 kcal/mol; (L) 16 : 0/16 : 0-phosphatidylethanolamine: T_c 60 °C, ΔH 8.5 kcal/mol.

with a ΔH value that is almost the same. 18 : 1_{tr}/18 : 1_{tr}-phosphatidylethanolamine also behaves in this way. The unsaturated 18 : 1_c/18 : 1_c- and 16 : 1_c/16 : 1_c-phosphatidylethanolamine, for which no transition temperatures were reported before undergo a transition at approximately the same temperature as do the corresponding phosphatidylcholines but the ΔH values are strongly decreased as compared to the phosphatidylcholines. Other than the transition at —16 °C and —33.5 °C for 18 : 1_c/18 : 1_c- and 16 : 1_c/16 : 1_c-phosphatidylethanolamine respectively (Fig. 1) no other transitions were observed between —50 °C and +70 °C. However, when we studied these cis-unsaturated phosphatidylethanolamines by freeze-fracture electron microscopy quite distinct changes in morphology at other temperatures than the calorimetric transition temperatures could be observed. In 18 : 1_c/18 : 1_c-phosphatidylethanolamine a conversion from a lamellar to a hexagonal phase (Fig. 2) could be observed when the

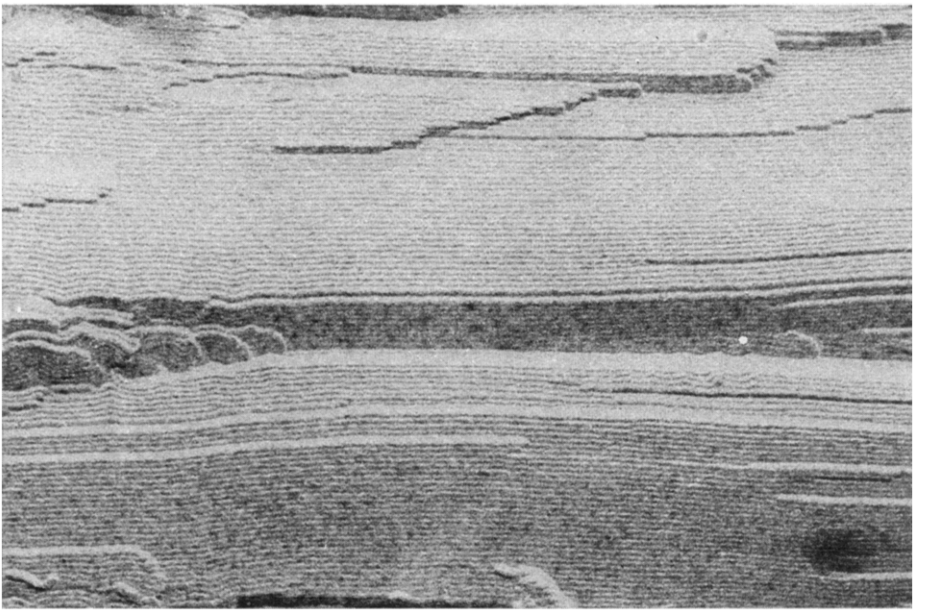

Fig. 2. Freeze-fracture electron micrograph of a hexagonal structure observed in a 18 : 1_c/18 : 1_c-phosphatidylethanolamine. The sample was quenches from 20 °C. Magnification 100 000 ×.

temperature was raised to about 20 °C. In 16 : 1_c/16 : 1_c-phosphatidylethanolamine this change in structure was observed around 0 °C. These changes are in agreement with ^{31}P-NMR data obtained on these lipids [8], where a similar phase change was observed. Hexagonal phases in naturally occurring phosphatidylethanolamines were reported before in X-ray studies [17]. Thus, gross structural alterations appear to occur in phosphatidylethanolamine-water systems at temperatures where calorimetry could not detect a transition.

Before studying the effect of cholesterol in mixtures of phosphatidylethanolamines and phosphatidylcholines it is necessary to know whether there are differences between the phosphatidylethanolamine/cholesterol and the phosphatidylcholine/cholesterol interactions. In Fig. 3 it can be seen that both in a saturated and in an unsaturated phosphatidylethanolamine introduction of cholesterol gradually abolishes the transition peak. Another observation is that the transition peak both for the 16 : 1_c/16 : 1_c- and the 14 : 0/14 : 0-phosphatidylethanolamine is shifted to lower temperatures by cholesterol, this shift is more pronounced than with synthetic phosphatidylcholines [1–3]. When the enthalpy changes of the transition were plotted against the ratio of cholesterol to phospholipid straight lines could be obtained intersecting the abcissa at a ratio of about 0.5. This result demonstrates that for phosphatidylethanolamine/cholesterol and for phosphatidylcholine/cholesterol mixtures a similar calorimetric behaviour is observed.

Binary mixtures of phospholipids can exist in two distinct forms, those who show cocrystallization and those which show monotectic behaviour (phase separation). In mixtures, showing cocrystallization a possible specificity of cholesterol is deduced from a change in the temperature of the midpoint of the transition after

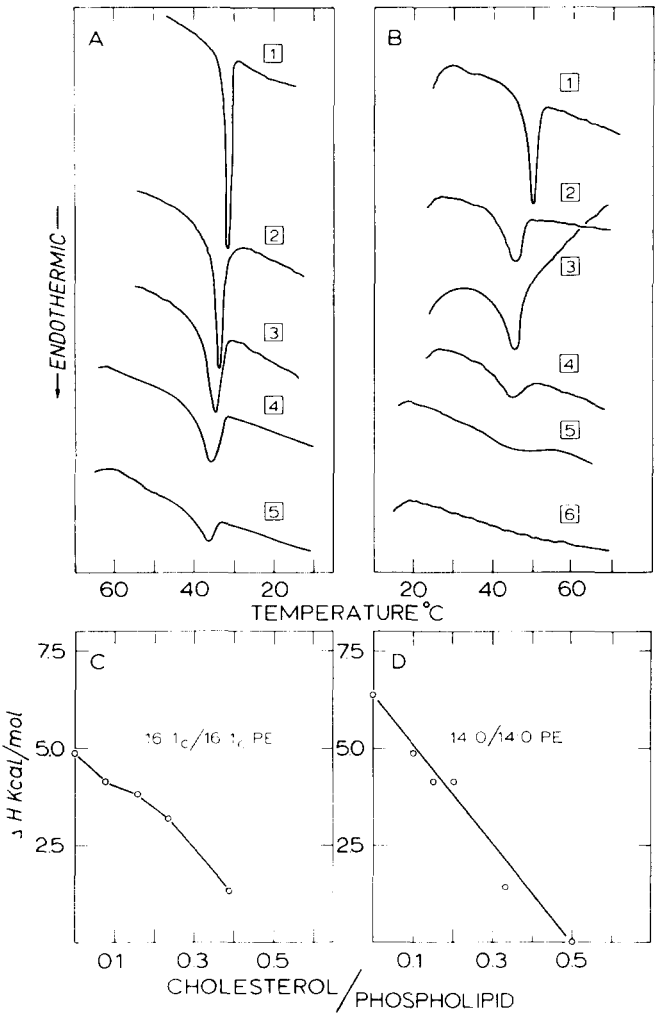

Fig. 3. The influence of increasing amounts of cholesterol on the lipid phase transition of a saturated and an unsaturated phosphatidylethanolamine (PE). Heating scans are shown. A. 16 : 1_c/16 : 1_c-phosphatidylethanolamine. Molar ratios of cholesterol to phospholipid (1) 0.000 (2) 0.078 (3) 0.155 (4) 0.232 (5) 0.387. B. 14 : 0/14 : 0-phosphatidylethanolamine. Molar ratios of cholesterol to phospholipid: (1) 0.00 (2) 0.10 (3) 0.15 (4) 0.20 (5) 0.33 (6) 0.50. C and D. Plots of the ΔH values calculated from the thermograms in A and B respectively versus the molar ratio of cholesterol to phospholipid.

addition of cholesterol [5]. Because cholesterol already considerably shifts the phase transition temperature of pure phosphatidylethanolamines a clear interpretation of such data would be very difficult. Therefore only mixtures showing phase separation were studied; in these cases interaction of cholesterol can be deduced from the ΔH value of the lipid phase transitions. It has been shown [4, 5] that in monotectic mixtures of phosphatidylcholines, cholesterol up to 20 mol% preferentially reduced the transition peak of the phosphatidylcholine with the lowest transition temperature,

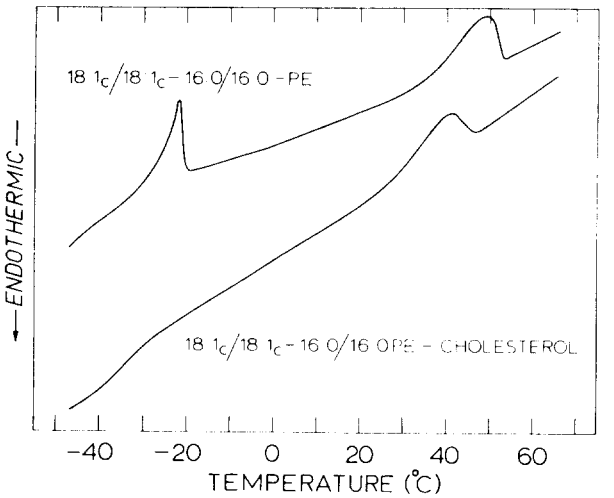

Fig. 4. The influence of cholesterol on the thermotropic behaviour of an equimolar mixture of 18 : 1_c/18 : 1_c- and 16 : 0/16 : 0-phosphatidylethanolamine (PE). Cooling scans are shown. ΔH values without cholesterol 4.6 and 8.7 kcal/mol and in the presence of 16.5 mol% of cholesterol 0.7 and 8.4 kcal/mol for the transitions of 18 : 1_c/18 : 1_c- and 16 : 0/16 : 0-phosphatidylethanolamine respectively.

which implies that cholesterol is frozen out of the gel phase patches in the bilayer where the upper component is concentrated [5]. How does cholesterol behave in monotectic mixtures of phosphatidylethanolamines? To investigate this we prepared an equimolar mixture of 18 : 1_c/18 : 1_c- and 16 : 0/16 : 0-phosphatidylethanolamine. In Fig. 4 the cooling scan of this mixture is shown. Two well resolved transitions can be seen with ΔH values of 4.6 and 8.7 kcal/mol for the 18 : 1_c/18 : 1_c- and 16 : 0/16 :0-phosphatidylethanolamine peaks respectively. Introduction of 16.5 mol% of cholesterol led to a broadening of the lower transition and a decrease in the enthalpy change from 4.6 to 0.7 kcal/mol. The upper transition was somewhat shifted to lower temperatures but the ΔH value was not affected (8.4 kcal/mol). This shift of the upper transition was also noted in monotectic mixtures of phosphatidylcholines [4, 5]. From the above experiment it can be concluded that also in monotectic mixtures of phosphatidylethanolamines cholesterol preferentially interacts with the component with the lowest transition temperature.

In Fig. 5 two typical examples of monotectic phosphatidylcholine/phosphatidylethanolamine mixtures are shown in which levels of cholesterol lower than 33 mol% were introduced. In a mixture of 18 : 1_c/18 : 1_c-phosphatidylcholine and 14 : 0/14 : 0-phosphatidylethanolamine (Fig. 5A) the phosphatidylethanolamine peak is shifted to a higher temperature than that of the pure component. At the moment this is not understood because normally the transition of the higher melting compound is shifted to lower temperatures due to the presence of the liquid-crystalline lower melting compound. From the broadening of the two peaks and the shift it can be deduced that both phospholipids are present in the same bilayers and do not form separate structures. Introduction of 8.3 mol% of cholesterol led to a decrease of the heat of the transition of the phosphatidylcholine peak whereas no decrease was appa-

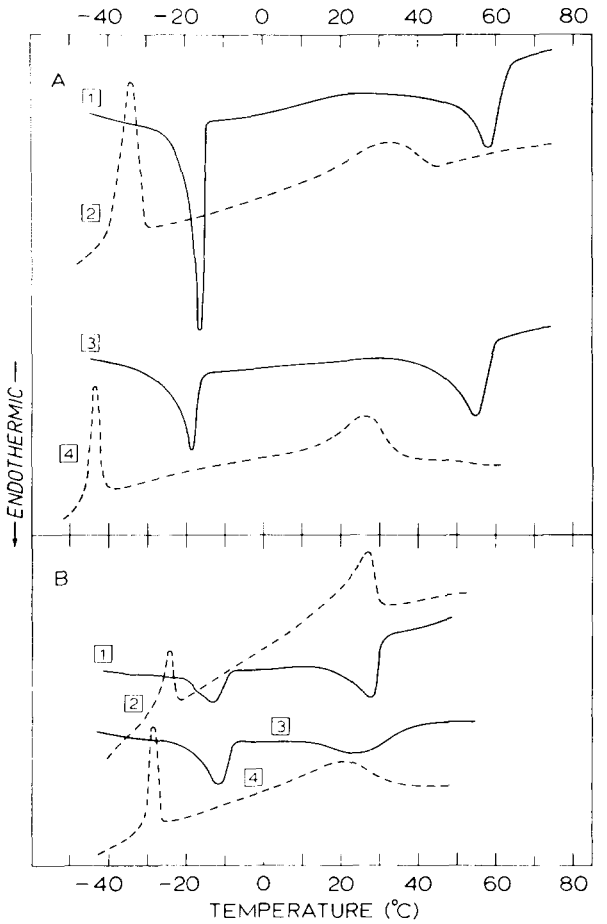

Fig. 5. The influence of cholesterol on the thermotropic behaviour of mixtures of lecithin and phosphatidylethanolamine. A. (1) Heating scan of a mixture of 2.26 μmol of 18 : 1_c/18 : 1_c-phosphatidylcholine and 2.02 μmol of 14 : 0/14 : 0-phosphatidylethanolamine. (2) Cooling scan of the same mixture. ΔH values 10.5 and 6.3 kcal/mol for the transition of 18 : 1_c/18 : 1_c-phosphatidylcholine and 14 : 0/14 : 0-phosphatidylethanolamine respectively. (3) Heating scan for a mixture of 1.75 μmol of 18 : 1_c/18 : 1_c-phosphatidylcholine and 1.56 μmol of 14 : 0/14 : 0-phosphatidylethanolamine in the presence of 8.3 mol% cholesterol. (4) Cooling scan of the mixture. ΔH values 6.0 and 5.8 kcal/mol for the transitions of 18 : 1_c/18 : 1_c-phosphatidylcholine and 14 : 0/14 : 0-phosphatidylethanolamine respectively. B. (1) Heating scan of a mixture of 1.80 μmol of 18 : 1_c/18 : 1_c-phospatidyl ethanolamine and 2.03 μmol of 16 : 0/16 : 0-phosphatidylcholine. (2) Cooling scan of the mixture. ΔH values 4.5 and 8.4 kcal/mol for the transitions of 18 : 1_c/18 : 1_c-phosphatidylethanolamine and 16 : 0/16 : 0-phosphatidylcholine respectively. (3) Heating scan of a mixture of 2.10 μmol of 18 : 1_c/18 : 1_c-phosphatidylethanolamine and 2.37 μmol of 16 : 0/16 : 0-phosphatidylcholine in the presence of 21.4 mol% of cholesterol. (4) Cooling scan of the mixture. ΔH values 4.4 and 2.1 kcal/mol for the transitions of 18 : 1_c/18 : 1_c-phosphatidylethanolamine and 16 : 0/16 : 0-phosphatidylcholine respectively.

TABLE I

THE EFFECT OF CHOLESTEROL ON THE HEAT CONTENTS OF THE TRANSITIONS OCCURRING IN DIFFERENT MONOTECTIC PHOSPHOLIPID MIXTURES

Mixture	Molar-ratio (mol/mol)	Cholesterol content (mol%)	ΔH lower melting component in the mixture (kcal/mol)	ΔH higher melting component in the mixture (kcal/mol)
18 : 1_c/18 : 1_c-PE/16 : 0/16 : 0-PE	50 : 50	0.0	4.6 ± 0.5	8.7 ± 0.9
18 : 1_c/18 : 1_c-PE/16 : 0/16 : 0-PE	50 : 50	16.5	0.7 ± 0.2	8.4 ± 0.8
16 : 1_c/16 : 1_c-PC/14 : 0/14 : 0-PE	51 : 49	0.0	8.8 ± 0.9	6.7 ± 0.7
16 : 1_c/16 : 1_c-PC/14 : 0/14 : 0-PE	51 : 49	16.1	0.0	6.1 ± 0.5
18 : 1_c/18 : 1_c-PC/14 : 0/14 : 0-PE	48 : 52	0.0	10.5 ± 0.8	6.3 ± 0.6
18 : 1_c/18 : 1_c-PC/14 : 0/14 : 0-PE	48 : 52	8.4	6.0 ± 0.5	5.8 ± 0.6
16 : 1_c/16 : 1_c-PE/14 : 0/14 : 0-PC	50 : 50	0.0	3.8 ± 0.4	7.0 ± 0.6
16 : 1_c/16 : 1_c-PE/14 : 0/14 : 0-PC	50 : 50	13.1	3.8 ± 0.3	2.0 ± 0.2
16 : 1_c/16 : 1_c-PE/16 : 0/16 : 0-PC	52 : 48	0.0	4.1 ± 0.4	8.1 ± 0.8
16 : 1_c/16 : 1_c-PE/16 : 0/16 : 0-PC	52 : 48	14.4	4.0 ± 0.3	4.6 ± 0.4
18 : 1_c/18 : 1_c-PE/14 : 0/14 : 0-PC	51 : 49	0.0	4.5*	6.8*
18 : 1_c/18 : 1_c-PE/14 : 0/14 : 0-PC	51 : 49	13.9	5.1 ± 0.5	1.7 ± 0.2
18 : 1_c/18 : 1_c-PE/16 : 0/16 : 0-PC	47 : 53	0.0	4.2 ± 0.3	8.4 ± 0.7
18 : 1_c/18 : 1_c-PE/16 : 0/16 : 0-PC	47 : 53	21.1	4.4 ± 0.4	2.1 ± 0.2

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

* ΔH values of the pure components because in the mixture no complete phase separation is found.

rent in the transition of the phosphatidylethanolamine. In contrast, in a mixture of 18 : 1_c/18 : 1_c-phosphatidylethanolamine and 16 : 0/16 : 0-phosphatidylcholine (Fig. 5) introduction of 21.1 mol% cholesterol did not affect the ΔH of the lower melting component, the phosphatidylethanolamine, but led to a decrease in the enthalpy change of the transition of the higher melting component, the phosphatidylcholine. From all other mixtures which were studied, both in cases where the phosphatidylcholine was the lowest and where it was the highest melting component, comparable data could be obtained which are summarized in Table I.

A more quantitative description of the cholesterol preference is shown in Fig. 6. In mixtures of 18 : 1_c/18 : 1_c-phosphatidylethanolamine and 16 : 0/16 : 0-phosphatidylcholine increasing amounts of cholesterol were introduced. Cooling curves are shown in the upper part of Fig. 6. For each of the peaks the ΔH value was calculated and plotted versus the molar ratio of cholesterol and the total phospholipid content (lower parts of Fig. 6). The plots show that up to a 0.2 molar ratio there is a gradual decrease in the energy content of the phase transition of the phosphatidylcholine, which is the upper melting component, whereas the ΔH of the phosphatidylethanolamine is not decreasing. Above a 0.3 molar ratio the phosphatidylethanolamine is also affected. In this region the ΔH of the phosphatidylcholine is still not zero; it keeps decreasing until, at near the 0.5 ratio (33 mol% of the cholesterol), both transitions are abolished completely.

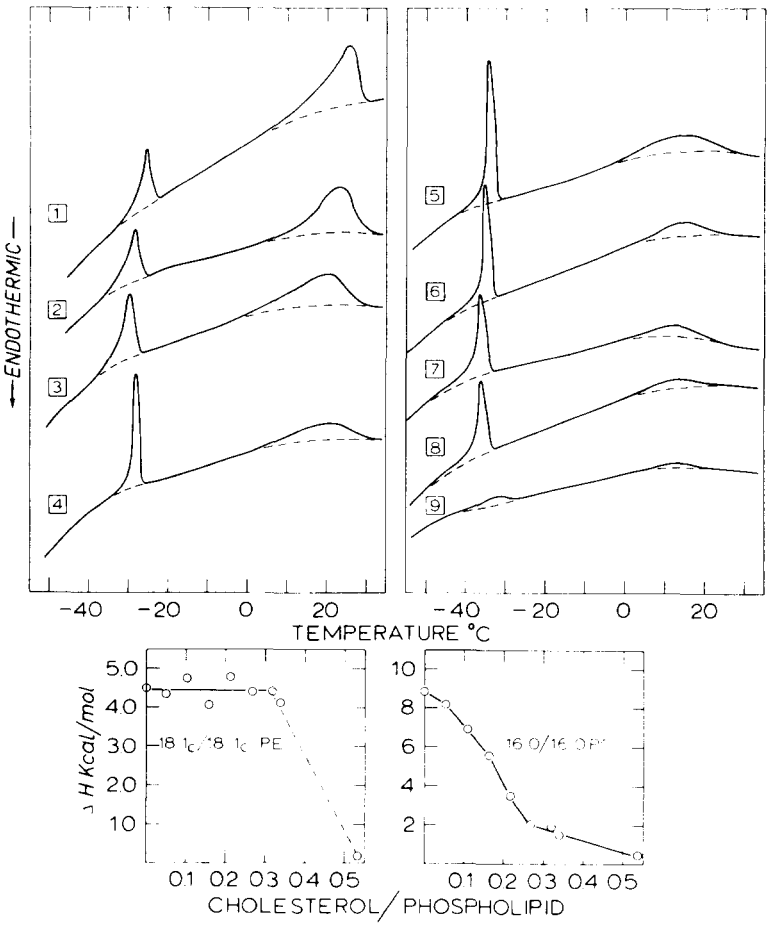

Fig. 6. The influence of increasing amounts of cholesterol on the lipid phase transitions in an equimolar mixture of 18 : 1_c/18 : 1_c-phosphatidylethanolamine (PE) and 16 : 0/16 : 0-phosphatidylcholine (PC). In the upper part of the figure cooling scans are shown. Cholesterol to phospholipid ratios in the mixtures 1–9. (1) 0.000 (2) 0.053 (3) 0.107 (4) 0.160 (5) 0.214 (6) 0.267 (7) 0.340 (8) 0.340 (9) 0.535. In the lower part of the figure the ΔH values of the two lipid transitions calculated from the scans in the upper part of the figure are plotted versus the cholesterol to phospholipid ratio.

DISCUSSION

Saturated or trans-unsaturated phosphatidylethanolamines undergo a lipid phase transition some 20–30 °C higher than the corresponding phosphatidylcholine. It is known that phosphatidylethanolamines, compared to phosphatidylcholines, have a small polar head group which is thought to be oriented tangential to the plane of the bilayer [18]. This small head group allows a very close packing of the phosphatidylethanolamine molecules. There is also less water penetration into the polar head region [20]. The difference in molecular packing is reflected in the elevated transition temperature of these phosphatidylethanolamines compared to the phosphatidyl-

cholines. However, the transition temperatures of cis-unsaturated phosphatidylethanolamines fall in the same temperature region as the corresponding phosphatidylcholines but the ΔH values of the former are markedly reduced. This indicates that the presence of cis-double bonds in both chains may not allow that close molecular packing that can be achieved in the saturated species. It was noted before [19] that bound water determinations of unsaturated phosphatidylethanolamines led to higher values than with saturated phosphatidylethanolamines which is in agreement with a more loose packing of the unsaturated phosphatidylethanolamine molecules.

The results of this study show that with the use of differential scanning calorimetry, no differences can be found in the interactions of phosphatidylethanolamines with cholesterol compared to those of phosphatidylcholines with cholesterol, at least in the pure systems. Also in monotectic mixtures of phosphatidylethanolamines no differences were seen compared to the findings in phosphatidylcholine mixtures. Here cholesterol also preferentially reduces the cooperative phase transition of the lowest melting component in the mixture which indicates that cholesterol, at temperatures where phase separation occurs, is excluded from the gel phase patches in the bilayer, where the lipid with the highest transition temperature in the mixture is concentrated.

In monotectic mixtures of phosphatidylethanolamines and phosphatidylcholines, regardless of what is the lowest melting component, cholesterol is preferentially associated with the phosphatidylcholine molecules present in the mixture. This strongly suggests that this preference of cholesterol for the phosphatidylcholine molecules has to exist also at temperatures where both phospholipid components are in the liquid-crystalline state. As most mammalian plasma membranes contain both phosphatidylcholine and phosphatidylethanolamine as well as cholesterol this preference of phosphatidylcholine over phosphatidylethanolamine may have a biological significance.

The phospholipid components of the different biomembranes have been shown to be asymmetrically distributed over the two sides of the membrane [21, 22]. Phosphatidylcholine and sphingomyelin are major phospholipids present at the outer monolayer whereas phosphatidylethanolamine and phosphatidylserine are predominantly found at the inner leaflet of the membrane. From recent experiments on the human red cell membrane it was suggested that more cholesterol is found in the outer than in the inner layer of the membrane [23].

Preferential interactions of cholesterol with certain phospholipid classes as described in this study for phosphatidylcholine and phosphatidylethanolamine may be a mechanism leading to such an asymmetric distribution of cholesterol. This possibility has to be considered as welll as that of a difference in unsaturation of both sides as was suggested before [22, 24].

More definite conclusions in this respect have to await further studies with other phospholipid combinations.

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REFERENCES

1 Ladbrooke, B. D., Williams, R. M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
2 De Kruijff, B., Demel, R. A. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 255, 331–347
3 Hinz, H. J. and Sturtevant, J. M. (1972) J. Biol. Chem. 247, 3697–3700
4 De Kruijff, B., Demel, R. A., Slotboom, A. J., van Deenen, L. L. M. and Rosenthal, A. F. (1973) Biochim. Biophys. Acta 307, 1–19
5 De Kruijff, B., van Dijck, P. W. M., Demel, R. A., Schuijff, A., Brants, F. and van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 356, 1–7
6 Lee, A. G. (1976) FEBS Lett. 62, 359–363
7 van Deenen, L. L. M. and de Haas, G. H. (1964) Adv. Lipid Res. 2, 168–229
8 Cullis, P. R. and de Kruijff, B. (1976) Biochim. Biophys. Acta 436, 523–540
9 Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468
10 Ververgaert, P. H. J. Th., de Kruijff, B., Verkley, A. J. and van Deenen L. L. M. (1975) Chem. Phys. Lipids 14, 97–101
11 Van Dijck, P. W. M., Ververgaert, P. H. J. Th., Verkley, A. J., van Deenen, L. L. M. and de Gier, J. (1975) Biochim. Biophys. Acta 406, 465–478
12 Ververgaert, P. H. J. Th., Verkley, A. J., Elbers, P. F. and van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 311, 320–329
13 Ladbrooke, B. D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–367
14 Chapman, D., Urbina, J. and Keough, K. M. (1974) J. Biol. Chem. 249, 2512–2521
15 Blume, A. and Ackermann, Th. (1974) FEBS Lett. 43, 71–74
16 Vaughan, D. J. and Keough, K. M. (1974) FEBS Lett. 47, 158–160
17 Reiss-Husson, F. (1967) J. Mol. Biol. 25, 363
18 Verkley, A. J., Ververgaert, P. H. J. Th., de Kruijff, B. and van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 373, 495–501
19 Phillips, M. C., Finer, E. G. and Hauser, H. (1972) Biochim. Biophys. Acta 290, 397–402
20 Jendrasiak, G. L. and Mendible, J. C. (1976) Biochim. Biophys. Acta 424, 149–158
21 Verkley, A. J., Zwaal, R. F. A., Roelofsen, B., Comfurius, P., Kastelijn, D. and van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 323, 178–193
22 Emmelot, P. and van Hoeven, R. P. (1975) Chem. Phys. Lipids 14, 236–246
23 Fisher, K. A. (1976) Proc. Natl. Acad. Sci. U.S. 73, 173–177
24 Van Dijck, P. W. M., van Zoelen, E. J. J., Seldenrijk, R., van Deenen, L. L. M. and de Gier, J. (1976) Chem. Phys. Lipids, in the press