Biochimica et Biophysica Acta, 356 (1974) 1–7

BBA 76680

NON-RANDOM DISTRIBUTION OF CHOLESTEROL IN PHOSPHATIDYLCHOLINE BILAYERS

B DE KRUYFF[a], P W M VAN DIJCK[a], R. A DEMEL[a], A SCHUIJFF[b], F BRANTS[b] and L L M VAN DEENEN[a]

[a]*Laboratory of Biochemistry and* [b]*Laboratory of Crystal Chemistry, State University of Utrecht, Utrecht (The Netherlands)*

(Received January 18th, 1974)

SUMMARY

1. The effect of cholesterol upon the phase transition occurring in various mixtures of synthetic disaturated phosphatidylcholines with fatty acid constituents of 12, 14, 16, and 18 carbon atoms was measured by differential scanning calorimetry.

2. Mixtures which differ 2 carbon atoms show cocrystallisation of the paraffin chains. In these mixtures cholesterol interacts randomly with the various phosphatidylcholine species.

3. Mixtures which differ 4 or more carbon atoms show monotectic behaviour (phase separation). In these mixtures chlolesterol interacts preferentially with the phosphatidylcholine species with the lowest transition temperature. This results in a non-random distribution of cholesterol at temperatures at which phase separation occurs. The implications of these findings for cholesterol-containing biological membranes are discussed.

INTRODUCTION

Cholesterol is a major constituent of many biological membranes. Although its exact function is as yet not understood, many studies have indicated that it can affect the packing of the paraffin chains of lipids in model and biological membranes (see review of Phillips [1]). The physical chemical state of the lipids is of critical importance for the cholesterol–lipid interaction. When the lipids are in the liquid–crystalline state cholesterol decreases the chain mobility [2, 3] and reduces the mean molecular area [4–6]. When the lipids are in the gel state cholesterol increases the chain mobility [3, 7, 8]

As yet there is almost nothing known about whether cholesterol will show a preference for any of the phospholipid species present in membranes composed of various molecular species of lipids. We therefore investigated systematically the effect of cholesterol upon the phase transition(s) occurring in various mixtures of synthetic phosphatidylcholines by differential scanning calorimetry. Mixtures showing cocrystallization and also mixtures showing monotectic behaviour (solid phase immiscibility) were analysed. It is well known that the interaction of cholesterol with lipids in model [9, 12] and biological [10, 13] membranes causes a decrease in the energy content of the phase transition of these lipids.

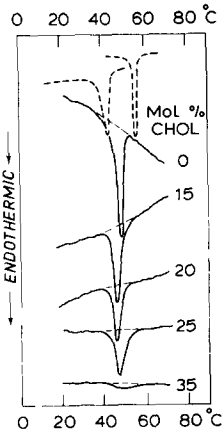
EXPERIMENTAL

1,2-Dilauryl-*sn*-glycero-3-phosphorylcholine (12 0/12 0-phosphatidylcholine), 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (14 0/14 0-phosphatidylcholine), 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (16 0/16 0-phosphatidylcholine) and 1,2-distearoyl-*sn*-glycero-3-phosphorylcholine (18 0/18 0-phosphatidylcholine) were synthesized as described before [14] Cholesterol (Fluka, Buchs, Switzerland) was recrystallised twice from ethanol All lipids were chromatographically pure

Calorimetric experiments were performed on a Perkin–Elmer DSC-2B apparatus operating at a heating rate of 5 °C/min at range 0 5 as described previously [10, 11] 0 3 ml of a chloroform solution containing 7 μmoles phosphatidylcholine with or without cholesterol was evaporated in a 1-ml test tube to dryness Residual solvent was removed by storing the tube overnight under high vacuum 50 μl water–glycol (1 · 1, v/v) was added The lipids were dispersed by agitating on a vortex mixer for 1 min above the transition temperature of the lipids About 15 μl of this dispersion was sealed in an aluminium sample pan After the calorimetric scans the amount of phosphatidylcholine present in the pan was determined by a phosphorus determination [15] Each sample was scanned at least 4 times to show the complete reversibility of the transitions Storing the sample at room temperature up to 24 h did not significantly influence the temperatures and energy contents of the transitions The variation in the transition temperature between various scans was 1 C or less The maximal variation in the determination of the energy content of the phase transition was 10 % Only heating curves are presented in this study The pre-transitional endotherms which were previously [9, 16, 25] observed in several phosphatidylcholines were not found in this study This might be caused by the presence of glycol in the lipid dispersion The temperatures and energy contents of the main transition were not affected by the presence of glycol

RESULTS AND DISCUSSION

Lipid bilayers composed of equimolar amounts of 12 0/12 0-phosphatidylcholine–14 0/14 . 0-phosphatidylcholine, 14 · 0/14 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine and 16 0/16 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine show cocrystallization of the paraffin chains [16–18] This is indicated in Fig 1 for the 16 0/16 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture One endothermic peak is observed at a temperature intermediate between the transition temperatures of the individual phosphatidylcholines Increasing amounts of cholesterol reduce the energy content of the phase transition (Figs 1 and 2) just as has been reported for bilayers composed of only one phospholipid species [9–11] At cholesterol concentrations between 30 and 40 mole% the transition vanished Apparently all phosphatidylcholine molecules are interacting with cholesterol at these concentrations Previously observed values for the percentage of cholesterol eliminating the phase transition of various phosphatidylcholine species also fall in this range [10, 12] Hinz and Sturtevant [12] suggested that each cholesterol molecule added removes 2 molecules of lipid from the cooperative melting gel phase, resulting in a complete disappearance of this phase at 33 3 mole% cholesterol



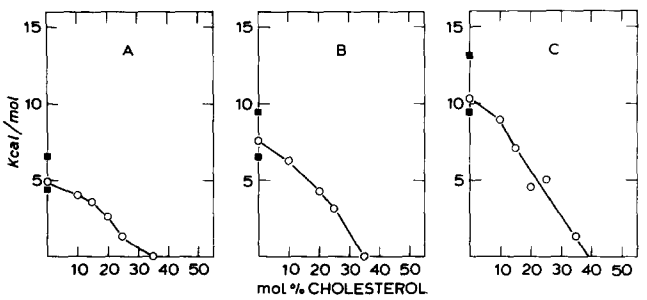
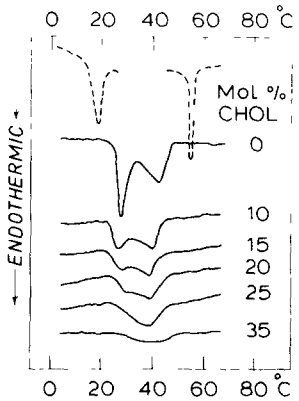


Fig 1 Calorimetric scans of the equimolar 16 0/16 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture containing increasing percentages of cholesterol The dotted curves represent the phase transitions of pure 16 0/16 0-phosphatidylcholine and pure 18 0/18 0-phosphatidylcholine

Fig 2 Effect of cholesterol upon the energy contents of the phase transition occurring in the equimolar mixtures (A) 12 0/12 . 0-phosphatidylcholine–14 0/14 0-phosphatidylcholine (B), 14 0/14 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine (C) and 16 0/16 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine. (■) energy content of the phase transition of the pure phosphatidylcholines

The effect of cholesterol upon the thermotropic behaviour of mixtures of various phosphatidylcholines can give us information as to whether cholesterol interacts randomly or preferentially with one of the molecular species of phosphatidylcholine present. If the cholesterol in the mixture would interact specifically with one species we might expect a shift in the temperature of the transition to values closer to the transition of the phosphatidylcholine species which was not or was interacting to a lesser extent with cholesterol. On the other hand if cholesterol shows no preference for interaction with one of the species in the mixture the transition temperature of the mixture should not be greatly affected by the incorporation of increasing amounts of cholesterol in the lipid bilayer. Cholesterol might induce only a slight reduction (1–2 °C) of the temperature of the midpoint of the transition as was noted previously for membranes composed of a single phosphatidylcholine species [9–12] Fig 1 shows that cholesterol in the 16 : 0/16 . 0-phosphatidylcholine–18 · 0/18 · 0-phosphatidylcholine mixture does not significantly influence the temperature of the transition Cholesterol has also no effect upon the transition temperatures of the mixtures 12 · 0/12 0-phosphatidylcholine–14 . 0/14 . 0-phosphatidylcholine and 14 : 0/14 · 0-phosphatidylcholine–16 : 0/16 · 0-phosphatidylcholine (data not shown) This observation strongly suggests that cholesterol interacts randomly in a lipid bilayer composed of phosphatidylcholine species which show cocrystallization of the paraffin chains.

Bilayers composed of phosphatidylcholines with fatty acid chains differing at least 4 carbon atoms, show monotectic behaviour [16–18]. Figs 3–5 demonstrate this for equimolar mixtures of 14 : 0/14 · 0-phosphatidylcholine–18 : 0/18 : 0-phosphatidylcholine, 12 : 0/12 : 0-phosphatidylcholine–16 : 0/16 · 0-phosphatidylcholine,



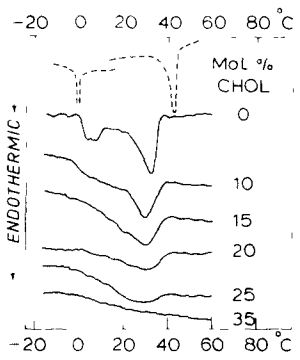


Fig 3 Calorimetric scans of the equimolar 14 0/14 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture containing increasing percentages of cholesterol The dotted curves represent the phase transitions of pure 14 0/14 0-phosphatidylcholine and 18 0/18 0-phosphatidylcholine

Fig 4 Calorimetric scans of the equimolar 12 0/12 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine mixture containing increasing percentages of cholesterol The dotted curves represent the phase transitions of pure 12 0/12 0-phosphatidylcholine and 16 0/16 0-phosphatidylcholine

12 0/12 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine Two transitions are visible in these mixtures which indicate that phase separation occurs A comparison of Figs 3–5 reveals that when the difference in the transition temperatures of the individual phosphatidylcholines increases, the degree of phase separation also becomes greater Thus in the 14 · 0/14 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture, where the difference in transition temperatures of the two species is relatively small (35 °C) the phase separation is not complete. However, in the case of 12 0/12 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture where the difference in transition temperatures is 55 °C the two transitions are well separated To see whether cholesterol has a preference for one species in these mixtures we investigated the effect of cholesterol upon the thermotropic behaviour of these mixtures When cholesterol interacts equally with both species one would observe an identical reduction of both transitions but when cholesterol interacts specifically with one of the species a selective reduction of one transition can be expected In the 14 0/14 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture it is obvious that at low concentrations cholesterol reduces the transition of 14 0/14 0-phosphatidylcholine more than the transition of 18 0/18 0-phosphatidylcholine (Fig 3) The incompletion of the phase separation makes it difficult to give a quantitative description of this phenomenon Cholesterol reduces the energy content of the sum of the transitions occurring in this sample identical to that of the mixtures which show cocrystallization of the paraffin chains (cf. Figs 2 and 6A) At 35 mole% cholesterol all the phosphatidylcholine molecules are interacting with cholesterol In the 12 0/12 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine mixture cholesterol shows at low concentrations (20 mole%) a preference for 12 0/12 0-phosphatidylcholine, at higher concentrations the transition of 16 0/16 0-phosphatidylcholine is more affected (Fig 4) Because in the 12 · 0/12 0-phosphatidylcholine–16 0,

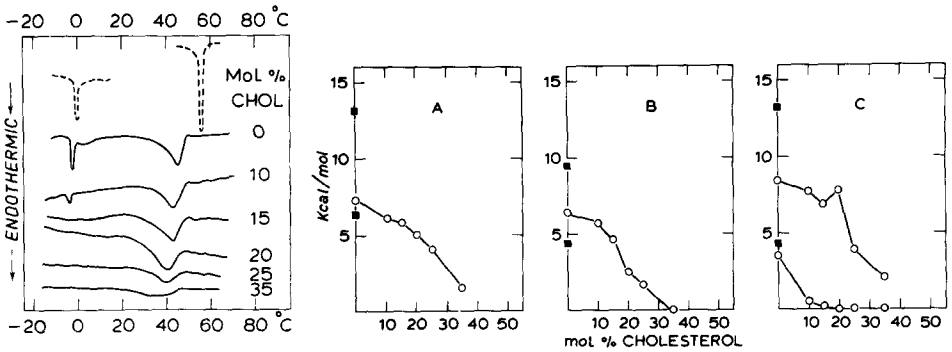


Fig 5 Calorimetric scans of the equimolar 12 0/12 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine mixture containing increasing percentages of cholesterol The dotted curve represent the phase transitions of pure 12 0/12 0-phosphatidylcholine and 18 0/18 0-phosphatidylcholine.

Fig 6 Effect of cholesterol upon the energy contents of the phase transition occurring in the equimolar mixtures 14 0/14 0-phosphatidylcholine–18 0/18 . 0-phospha'idylcholine (A), 12 0/12 · 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine (B), 12 0/12 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine (C) In A and B the energy content of the sum of the transitions is given In C the energy content of the individual transitions in the mixture (upper curve 18 0/18 0-phosphatidylcholine, lower curve 12 0/12 0-phosphatidylcholine) are given (■) energy content of the phase transition in the pure phosphatidylcholines

16 0-phosphatidylcholine mixture the phase separation is also not complete no quantitative measurement of this specific interaction can be given The overall reduction of the energy content of the transition is comparable with the other mixtures tested (Figs 2 and 6B) Because the phase separation is more complete in the 12 . 0/12 0-phosphatidylcholine–18 0/18 . 0-phosphatidylcholine mixture we can measure the energy content of both transitions (Figs 5 and 6C) Up to 20 mole% cholesterol the energy content of only the 12 0/12 0-phosphatidylcholine transition is reduced, such that at 20 mole% all the 12 : 0/12 . 0-phosphatidylcholine have interacted with cholesterol From 20–40 % cholesterol the energy content of the 18 0/18 0-phosphatidylcholine transition is reduced*. In a previous paper [11] we observed the same effect of cholesterol upon the phase transition occurring in an equimolar mixture of 18 · 1c/18 1c-phosphatidylcholine**–18 . 0/18 0-phosphatidylcholine In this mixture where both transitions are also well separated cholesterol interacts first with 18 1c/18 1c-phosphatidylcholine and at higher concentrations also with 18 0/18 0-phosphatidylcholine [11]. We have to emphasize that the energy contents of the phase transitions of the individual phosphatidylcholine species are reduced by cholesterol in a comparable way [10–12] Our results indicate that cholesterol, at concentrations up to 20 mole%, shows a preference for species with the lowest transition temperature in phosphatidylcholine mixtures which show phase separation From this we conclude that under these conditions cholesterol is non randomly distributed in the lipid bilayer at temperatures at which phase separation occurs. This non random distribution is maximal in the mixtures, which show complete phase separation

* In all mixtures which show phase separation we observed that cholesterol produced a small decrease of the transition temperature of the higher melting species

** 1 2-dioleoyl-*sn*-glycero-3-phosphorylcholine

(12 0/12 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine and 18 1c/18 1c-phosphatidylcholine–18 0/18 0-phosphatidylcholine), decreases when the separation becomes less (12 0/12 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine and 14 0/14 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine) and is absent when no phase separation occurs (12 0/12 0-phosphatidylcholine–14 0/14 0-phosphatidylcholine, 14 0/14 0-phosphatidylcholine–16 0/16 0-phosphatidylcholine and 16 0/16 0-phosphatidylcholine–18 0/18 0-phosphatidylcholine)

Biological implications

Membrane of mitochondria [20], nuclei [20], microsomes [20] and cholesterol grown *Acholeplasma laidlawii* cells [10] contain relatively low (5–16 mole%) amounts of cholesterol Phase transitions were observed in these membranes [10–24] demonstrating that cholesterol does not interact with all lipids present This indicates that if phase separation occurs in these membranes, cholesterol will be non randomly distributed in these membranes at temperatures in the phase transition

The erythrocyte and myelin membrane are two types of membranes with a high (50 mole% [22, 27]) cholesterol concentration. In previous calorimetric studies of Ladbrooke et al [26] on the myelin membrane it was noticed that in excess water apparently no phase transitions occur in the membrane whereas the total cholesterol free lipids and the dehydrated membrane showed endothermic transitions which were attributed to lipid phase transitions It was furthermore demonstrated that total erythrocyte lipids showed no transitions whereas the cholesterol free lipids showed a small endothermic transition [9] Since the lipid composition of these membranes is very complex and the phase transition of the intact membrane in excess water is very difficult to detect calorimetrically no conclusions could be drawn as to whether cholesterol interacts at random with the various lipids present in these membranes If all the cholesterol in these membranes is available for interaction with phospholipids this amount of cholesterol is sufficient to interact with all the lipid species present However, it is not excluded that part of the cholesterol might be interacting with membrane proteins. In a recent paper, London et al [19] observed an interaction of the Folch–Lees protein from myelin with cholesterol If we assume that this cholesterol can no longer interact with lipids then the amount of cholesterol present in these membranes which can interact with lipid is less than 50 mole% Since rather unsaturated lipids as well as very saturated lipids, such as the sulfolipids in myelin [21] and sphingomyelin [22] in erythrocytes, are found in these membranes it is possible that cholesterol preferentially interacts with the most liquid species present The results will therefore be a non random distribution of cholesterol in these membranes In this respect it is important to recall the X-ray studies of Caspar [23] who concluded that cholesterol is asymmetrically distributed in the myelin membrane

REFERENCES

1 Phillips, M C (1972) in Progress in Surface and Membrane Science (Danielli, J F Rosenberg, M D and Cadenhead, D A , eds) Vol 5, 139–221, Academic Press, New York
2 Hubbel, W L and McConnell, H M (1971) J Am Chem Soc 93, 314–326
3 Oldfield, E , Chapman, D and Derbyshire, W (1971) FEBS Lett 16, 102–106
4 De Bernard, L (1958) Bull Soc Chim Biol 40, 161–170

5 Demel, R A , Geurts van Kessel, W S M and Van Deenen, L L M (1972) Biochim Biophys Acta 266, 26–40
6 Rand, R P and Luzzati, V. (1968) Biophys J 8, 125–137
7 Oldfield, E and Chapman, D (1972) FEBS Lett 21, 302–306
8 Marsh, D and Smith, I. C. P (1973) Biochim Biophys Acta 298, 133–141
9 Ladbrooke, B D , Williams, R M and Chapman, D (1968) Biochim Biophys Acta, 150, 333–340
10 De Kruyff, B , Demel, R A and Van Deenen, L L M. (1972) Biochim Biophys Acta 255, 331–347
11 De Kruyff, B , Demel, R A , Slotboom, A J , Van Deenen, L L M and Rosenthal, A F (1973) Biochim Biophys Acta 307, 1–19
12 Hinz, H J. and Sturtevant, J M. (1972) J. Biol Chem 247, 3697–3699
13 Rottem, S , Cirillo, V P , De Kruyff, B , Shinitsky, M and Razin, S (1973) Biochim Biophys. 323, 509–519
14 Van Deenen, L L. M and De Haas, G H (1964) Adv Lipid Res 2, 168–229
15 Fiske, C H and SubbaRow, Y (1925) J Biol Chem 66, 375–379
16 Phillips, M C , Ladbrooke, B D and Chapman, D (1970) Biochim Biophys Acta 196, 35–43
17 Ververgaert, Ph J Th , Verkleij, A J , Elbers, P F and Van Deenen, L. L M (1973) Biochim Biophys Acta 311, 320–329
18 Shimshick, E J and McConnell, H M (1973) Biochemistry 12, 2351-2360
19 London, Y , Demel, R A , Geurts van Kessel, W S M , Zahler, P. and Van Deenen, L L M (1974) Biochim Biophys Acta 332, 69–84
20 Lasser, N. L and Clayton, R B (1966) J Lipid Res 7, 413–420
21 Demel, R A , London, Y , Geurts van Kessel, W S M , Vossenberg, F G A and Van Deenen, L L M (1973) Biochim Biophys Acta 311, 507–519
22 Van Deenen, L L M and De Gier, J (1964) in The Red Blood Cell (Bishop, C and Surgenor, D. M , eds) pp 234–307, Academic Press, New York
23 Caspar, D L D and Kirschner, D A (1971) Nature New Biol 321, 46–49
24 Blazyk, J F and Steim, J M (1972) Biochim Biophys Acta 266, 737–741
25 Hinz, H J. and Sturtevant, J M (1972) J Biol Chem 247, 6071–6075
26 Ladbrooke, B D , Jenkinson, T J , Kamat, V B and Chapman, D (1968) Biochim Biophys Acta 164, 101–109
27 Van Deenen, L L M (1965) in Progress in the Chemistry of Fats and Related Lipids (Holman, R , ed), Vol VIII, part I, Oxford, Pergamon Press