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INTERACTION BETWEEN 1,2-DIPALMITOYL-L-PHOSPHATIDYL CHOLINE AND CHOLESTEROL. DSC STUDY *

ALDO MARINO, ROBERTA CURINI

Institute of Analytical Chemistry, University of Rome, Rome (Italy)

TIZIANA PARASASSI

Institute of Physical Chemistry, University of Rome, Rome (Italy)

ALESSANDRO PIERUCCI

Second Medical Clinic, University of Rome, Rome (Italy)

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ABSTRACT

The thermal behaviour of 1,2-dipalmitoyl-L-phosphatidyl choline is examined together with that of a mixture of 1,2-dipalmitoyl-L-phosphatidyl choline and cholesterol to analyse the interactions between these two systems and to obtain useful information at the level of biological membrane bilayers.

INTRODUCTION

The bilayer structure of phospholipids in membrane cells is now generally accepted. The study of the many phenomena concerning the structure and function of the cellular membranes and of their components will grow enormously in the future, especially by the use of model systems based on the amphiphilic characteristics of the phospholipids which make it possible to obtain them as globules in water in which other components that are constituents of the cells can be included.

These systems, by the use of different techniques such as NMR, EPR, spectrofluorimetry, DSC, X-rays, and so on, led to an explanantion of the problems that have been correlated with natural cellular systems.

One of the main characteristics examined has been the possibility of structural change and of chemico-physical changes of the lipidic bilayer as a function of the chemical nature of the phospholipids present and of their interaction with different substances. In particular, it has been pointed out that the presence of cholesterol, a chemical species generally present in different proportions in cellular membranes, implies a dramatic change in the bilayer structure, whether concerning the hydrophobic part or the polar heads, with a consequent change of the chemico-physical properties of the bilayer.

The formation of the cholesterol—phospholipid complex gives rise to a variation in the permeability of the liposomes and also to changes in the surface and then in the thickness of the bilayers. This last phenomenon has

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been correlated with a conformational change of the polar heads of the phospholipid [1]. The presence of cholesterol together with phospholipids in the liposomes affects the water permeability because it assists the elimination of the sharp change in the speed of permeation of water near the gel—liquid crystal transition temperature. That discontinuity disappears for cholesterol concentrations higher than 30 mole % [2].

Nevertheless, no data are available concerning the thermal behaviour of systems consisting only of cholesterol and phospholipids to distinguish the separation of the effect due to the presence of water or of the other components from that concerning the association phenomena between the two cited species. Further, no data exist in the literature concerning hysteresis phenomena due to temperature of associated systems.

The thermal behaviour of 1,2-dipalmitoyl-L-phosphatidyl choline is examined in this paper together with that of a mixture of 1,2-dipalmitoyl-L-phosphatidyl choline and cholesterol to analyse the interactions between these two systems and to obtain useful information at the biological level.

EXPERIMENTAL

Instrumentation

The DSC curves were obtained using a Perkin-Elmer DSC-2 differential scanning calorimeter interfaced with a Tektronix 31 computer. The heating rate was 10°C min^{-1} and the atmosphere in the furnace consisted of dry nitrogen at a flow rate of 100 ml min^{-1}.

Reagents

The cholesterol and chloroform were supplied by Merck and the 1,2-dipalmitoyl-L-phosphatidyl choline by Sigma. The purity of the compounds was checked by thin layer chromatography.

Sample preparation

The 1,2-dipalmitoyl-L-phosphatidyl choline was weighed directly into the capsules and analysed by DSC. The 1,2-dipalmitoyl-L-phosphatidyl choline—cholesterol mixtures were obtained by preparing separate chloroform solutions of the two compounds and then mixing in the instrument pans appropriate volumes of the two solutions to give the desired percentage ratio of the compounds. The chloroform was then slowly evaporated in a nitrogen stream and then under vacuum for 24 h. The DSC curves of samples of 1,2-dipalmitoyl-L-phosphatidyl choline recovered from chloroform by evaporation of the solvent in a nitrogen stream and in vacuum for different times show that the solvent is completely removed after 24 h (Fig. 1).

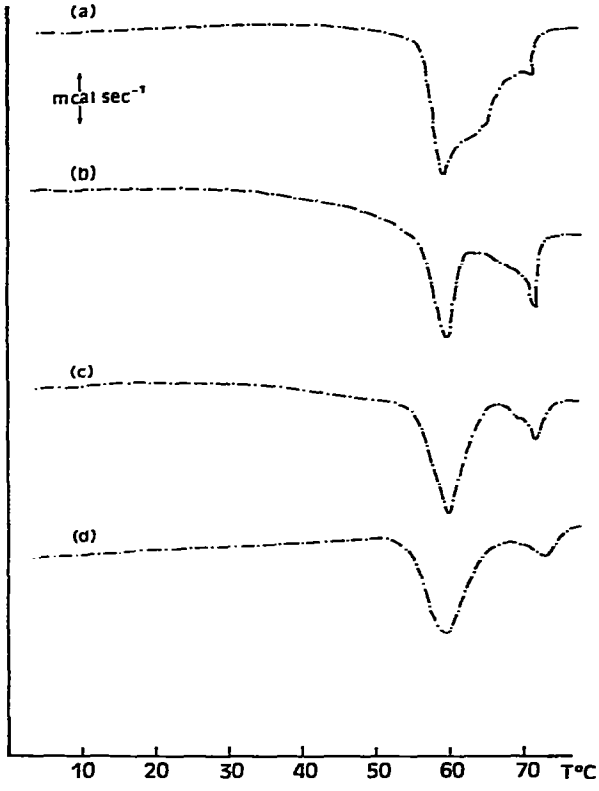

Fig. 1. DSC curves corresponding to samples of 1,2-dipalmitoyl-L-phosphatidyl choline dissolved in chloroform and analysed after standing under vacuum for (a) 1 h; (b) 6 h; (c) 18 h; (d) 24 h.

RESULTS

The DSC heating curve of 1,2-dipalmitoyl-L-phosphatidyl choline shows (Fig. 2) two unresolved endothermic transitions, the first of which is larger than the second, while there are no transitions in the cooling curve. On cycling the process between 10 and 80°C, no peaks appear during the second heating process, but on leaving the samples at room temperature for increasing times, the DSC heating slowly again takes on the original appearance (Fig. 3). Finally, if the sample after the first DSC heating is cooled to 50°C and held at that temperature for only 1 h, it again shows the original behaviour (Fig. 3).

The DSC curve of the phospholipids in the presence of 50 wt. % of water gives, according to Chapman and co-workers [3], two endothermic transitions shifted to lower temperatures and the area corresponding to the first is now smaller than that corresponding to the second.

Phospholipid—cholesterol mixtures containing increasing amounts of cholesterol were analysed by DSC (Fig. 4). The results show differences in the positions of the minima, or in the areas of the peaks, or in the ratios of the areas of the individual processes.

The DSC curves for pure phospholipids show that, on cycling the heat-

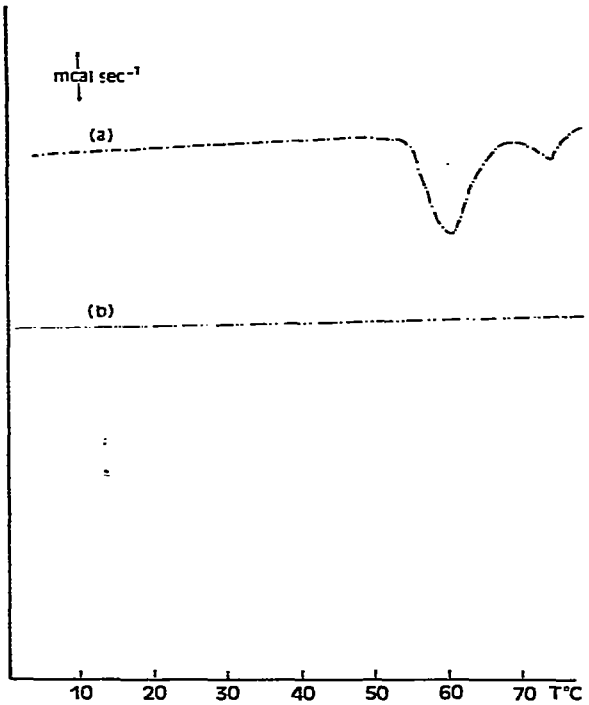

Fig. 2. DSC curves of 1,2-dipalmitoyl-L-phosphatidyl choline: (a) heating; (b) cooling. Heating rate: 10°C min^{-1}; atmosphere: nitrogen.

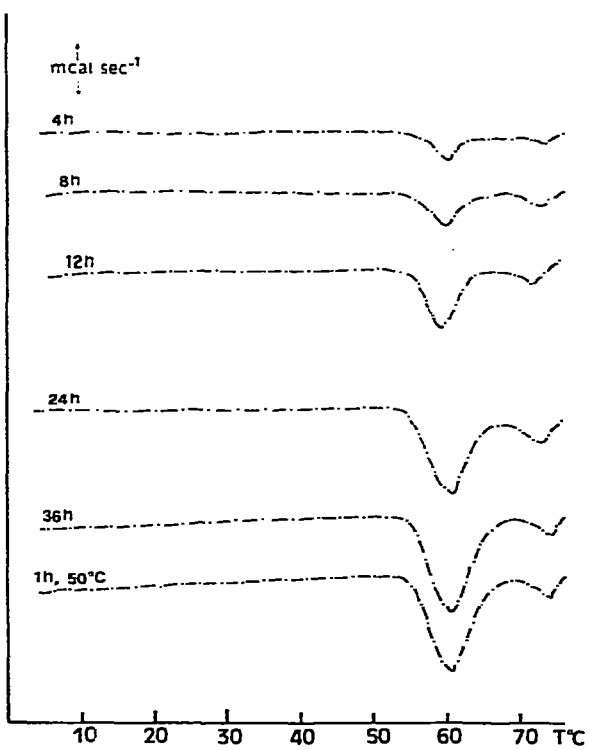

Fig. 3. DSC curves of 1,2-dipalmitoyl-L-phosphatidyl choline analysed at various times after a heating—cooling cycle.

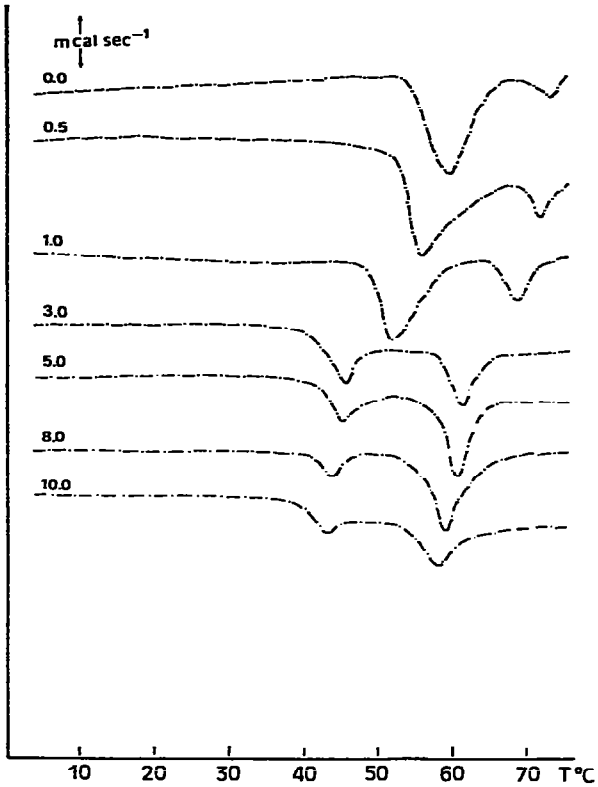

Fig. 4. DSC curves of samples of 1,2-dipalmitoyl-L-phosphatidyl choline containing various amounts of cholesterol.

ing—cooling process, the DSC curve corresponding to the second heating is flat. But if, after the first DSC heating, the sample is cooled to room temperature and kept at this temperature for increasing times, the positions of the minima and the areas of the peaks change with the time that the sample is kept at room temperature.

In some experiments, new peaks appear with very small areas and with the minima in anomalous positions. This phenomenon indicates that the initial equilibrium association state occurs very slowly and sometimes takes place as separate associations which correspond to the anomalous peaks.

The two unresolved endothermic transitions can be explained as being due to melting of the hydrocarbon chains in the phospholipid which is followed by a shortening phenomenon due to rolling up and to torsion. The hydrocarbon chains remain in a disordered condition for very long times at room temperature as shown by the DSC curves, which indicate that the original behaviour is restored only after 24 h. This brings us to the conclusion that the hydrocarbon chains of the phospholipid begin to flex at low temperatures. At the transition temperature, the hydrocarbon chains melt and it can be assumed that the chains, in spite of being bound at one end, take on a conformation similar to that of the molecules of a liquid paraffin.

In the case of the association of cholesterol with the phospholipids inside the cells, it is possible to discuss the hypothesis of Chapman et al. [4] in the light of the experimental data obtained.

(a) The phospholipid molecules, because of their amphiphilic nature, form oriented monolayers. The observed film depends on the solubility of the molecule in water and on the cohesion of the hydrocarbon chains and is then correlated with their tendency to melt.

(b) The tendency of a phospholipid to disperse in water is connected with the transition temperature of the hydrocarbon chains so that the unsaturated phospholipids, having lower transition temperatures, disperse more easily in water [5] than the saturated. The degree of fluidity of the chains is then directly related to the possibility of the phospholipid dispersing at body temperature, the importance of which is well known in the blood coagulation process, for example [6].

(c) The swelling process of the phospholipids, with consequent formation of the myelinic tubes, is a function of the transition temperature and occurs readily at body temperature with unsaturated phospholipids, but does not take place with saturated phospholipids.

(d) Attempts to obtain models of bilayer-type membranes gave good results only with unsaturated phospholipids [7]. In the case of the interaction between cholesterol and unsaturated phospholipids, the films obtained are more rigid [8] and, according to Chapman, these systems should have transition temperatures higher than those corresponding to the pure phospholipids.

The above considerations and the presence of cholesterol in natural cells lead to opposing conclusions, i.e. the presence of cholesterol should permit a higher fluidity of the hydrocarbon chains with a consequent lowering of the transition temperature of the system. According to Shah and Schulman [9], the apparent condensation of the mixed 1,2-dipalmitoyl-L-phosphatidyl choline—cholesterol monolayer can be explained by assuming the presence of molecular holes where the cholesterol is arranged and so there is no proportional increase in the area of the monolayer. The authors suggest that this phenomenon, rather than leading to condensation of the phospholipid, increases the fluidity of the hydrocarbon chains. Ladbrooke et al. [3], in a study of the system lecitin—cholesterol—water, suppose that the effect of cholesterol is to modify the ordered structure of the hydrocarbon chains of the phospholipid in the gel phase so that when cholesterol and lecitin are present in equimolecular ratio, all the chains are in a condition of fluidity. So, in a solid mixture, a cholesterol—lecitin interaction, such as to cause a lowering of the transition temperature, should be verified.

From the experimental data, it can be seen that

(a) the processes take place at a decreasing temperature as the cholesterol content increases, implying a higher fluidity of the hydrocarbon chains (Fig. 5);

(b) on increasing the cholesterol concentration, the DSC curves change, with a decrease in the area of the first peak and an increase in the area of the second, which becomes larger and flatter. When the cholesterol concentration becomes 10%, the behaviour of the curve is the same as that corresponding to the lecitin—water system;

(c) the total area of the process decreases with increasing cholesterol concentration until a cholesterol concentration of 50% is reached, at which

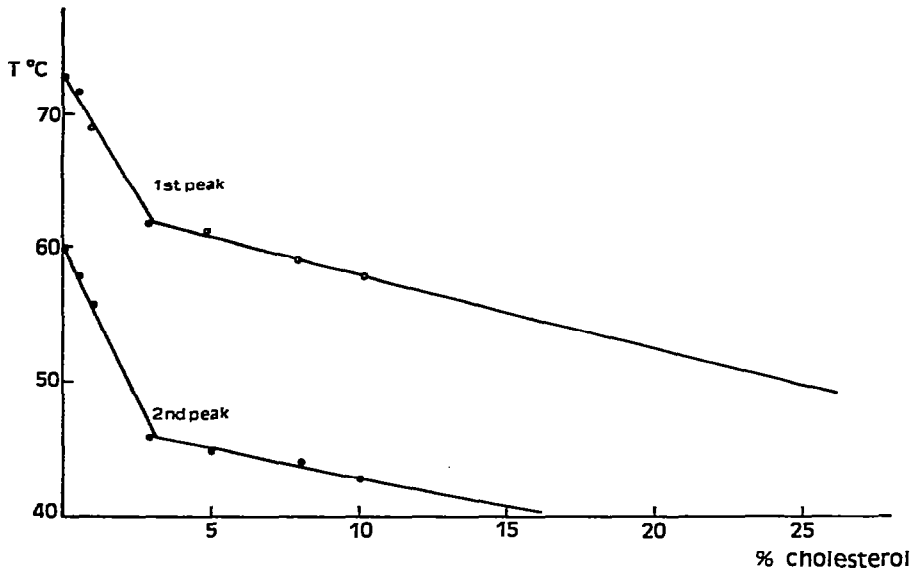

Fig. 5. Plot of the minima corresponding to samples of 1,2-dipalmitoyl-L-phosphatidyl choline containing various amounts of cholesterol vs. percent cholesterol.

point no instrumental signal is obtained.

From the data considered, it is concluded that cholesterol interacts with lecitin so as to increase the fluidity of the hydrocarbon chains.

It is supposed that the lecitin lamellae can rearrange the molecules of cholesterol so that the hexagonal packing of the lecitin is not modified, but only the cohesive strength between the hydrocarbon chains is reduced so that they gain a higher mobility with a consequent decrease of the transition temperature. A further increase in the cholesterol content then causes a change in the structural configuration of the phospholipid with a change of the charge distribution on the layer of polar heads with a consequent change in the fluidification of the hydrocarbon chains. This hypothesis then allows a maximum amount of cholesterol for which there is no change in the phospholipid structure.

The decrease in the temperature corresponding to the minima of the processes examined (Fig. 5) is represented by a line with a sharp change in slope at 3% cholesterol content which identifies the maximum content that can be tolerated without structural changes.

Finally, it is interesting to note that, in the DSC curves examined, the characteristic cholesterol endothermic transition which is localised at 37.5°C [10] never appears. This leads to the assumption that the phospholipid—cholesterol interaction at fluidification level is reciprocal.

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