Journal of Thermal Analysis, Vol. 38 (1992) 1775-1783

AC CALORIMETRIC STUDY OF PHASE TRANSITIONS IN PHOSPHATIDYLCHOLINE-CHOLESTEROL SYSTEMS

J. Hatta, M. Okada, S. Matuoka and H. Yao***

DEPARTMENT OF APPLIED PHYSICS, NAGOYA UNIVERSITY, NAGOYA 464–01, JAPAN
*DEPARTMENT OF PHYSICS, SAPPORO MEDICAL COLLEGE, SAPPORO 064, JAPAN
**DEPARTMENT OF PHYSICS, TOKYO INSTITUTE OF TECHNOLOGY, TOKYO 152, JAPAN

Using an ac calorimetric method, detailed behaviour of the heat capacity in dipalmitoylphosphatidylcholine-cholesterol system was studied in the cholesterol concentration less than 5 mol%. It was revealed that the heat capacity near the main transition was composed of at least four anomalies, i.e., multipeak took place in the heat capacity. This fact indicates that a simple theory explaining coexistence of two phases in two component systems does not work in the multipeak region. Then, relation between the multipeak heat capacity and the change of the ripple structure with the cholesterol concentration should be taken into account, when we consider thermodynamical behaviour of the systems.

Keywords: AC calorimetry, phase transition, phosphatidylcholine-cholesterol

Introduction

The phase transitions of phospholipid-cholesterol systems have been studied extensively by means of a variety of experiments [1–7]. So far phase diagram for these systems has been proposed. However, there still remain a lot of vague points, including whether these systems are able to be interpreted in terms of phase diagrams or not. In connection with the latter point, it is interesting to study at the low cholesterol concentrations, where the ripple repeat spacing changes as a function of cholesterol concentration [3].

Thermal measurement is one of the powerful tools in studying the phase transitions, Mabrey *et al.* [2] have carried out calorimetric measurement of dipalmitoylphosphatidylcholine-cholesterol systems using a differential scanning calorimeter. From the result it has been pointed out that the traces of thermogram are composed of the sum of two approximately symmetric peaks, a sharp one and

John Wiley & Sons, Limited, Chichester
Akadémiai Kiadó, Budapest

a broad one. The sharp peak shifts to slightly lower temperatures and becomes somewhat broader as the cholesterol concentration increases. The broad peak is centered at about 41.5°C up to 20 mol% cholesterol and then moves to about 46°C at 33 mol%. The enthalpies of the sharp peak decreases and then almost vanishes at 20 mol%. The enthalpies of the broad peak also decreases and diminishes at much high cholesterol concentrations. Independently Estep *et al.* [1] have performed the similar experiments. They have observed a sharp and a broad peak as well, however, they have decomposed the two peaks in a different way. The heat capacity appearing near the main transition has been interpreted by assuming that the line shape of the sample containing 24.2 mol% cholesterol is characteristic of the broad peak and that this peak keeps the same form at the lower cholesterol concentrations. Therefore, in the thermogram the high-temperature heat capacity trace was used to scale the magnitude of the broad peak, when the heat capacity at the low cholesterol concentrations was analyzed. The area of the broad peak was then subtracted from that of the total trace to obtain the area of the sharp peak at the low cholesterol concentrations. From the enthalpies thus obtained it appears that the sharp peak decreases linearly with increasing molar fraction of cholesterol, approaching zero at about 25 mol%. The broad peak increases with adding cholesterol until it reaches a maximum at about 25 mol%, and higher molar fractions of cholesterol results in a decrease of the broad peak.

For the similar system, ac calorimetric measurements have been made by Imaizumi and Hatta [4]. The results are almost consistent with those obtained with differential scanning calorimetry (DSC) [1, 2], but the resolution of the detailed trace is better in ac calorimetry than in DSC and therefore, it has clearly been shown that the heat capacity trace at the low cholesterol concentrations is composed of two distinct asymmetric sharp and broad peaks. Besides the calorimetric measurements, the observation of the ripple structure has been carried out with freeze-fracture electron microscopy [3]. It has been revealed that the inverse of the ripple repeat spacing decreases approximately linearly with the cholesterol concentration and approaches zero at about 20 mol%. Based upon the results of the freeze-fracture microscopy, it has been proposed that in adding cholesterol a microscopic phase separation happens in the ripple phase, i.e., the ripple strips in the dipalmitoylphosphatidylcholine-cholesterol systems consist of the alternative appearance of two regions, pure phospholipid and 20 mol% cholesterol ones. Taking into account the evidences obtained by ac calorimetry and also by freeze-fracture electron microscopy, the model has been extended as there are at least three microscopic regions, pure phospholipid one, 20 mol% cholesterol one and one with cholesterol less than 20 mol% [4]. In this case, the pure phospholipid region and the region with cholesterol less than 20 mol% causes the asymmetric sharp peak and the asymmetric broad peak, respectively, and on the other hand, the 20 mol% cholesterol region does not exhibit significantly anomalous behaviour near the main transition. These facts indicate that

the ripple phase is not a phase simply defined as so-called solid solution as expected thermodynamically but a phase accompanied with structural change as a function of cholesterol concentration.

Recently, Vist and Davis [7] have proposed the phase diagram from the experiments of deuterium nuclear magnetic resonance spectroscopy and differential scanning calorimetry. Let us focus our attention to their phase behaviour near the main transition: A narrow liquid-crystalline/gel phase coexistence region lies between 0 and 6 or more mol% cholesterol; there is an eutectic point between 7.5 and 10 mol% cholesterol; at the higher cholesterol concentrations a coexistence region lying between a sharp and broad peaks observed with DSC takes place. The experimental evidence related to the third points consistent with the results obtained by the former experiments. On the other hand, the first and the second points essentially new and important on considering the phase diagram further. In order to reexamine these facts, we will perform ac calorimetric experiments specially with paying attention to phase behaviour at the low cholesterol concentrations.

Experimental

Dipalmitoylphosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids Inc. and cholesterol from Sigma Chemicals Co. A proper amount of DPPC was dissolved in a stock solution of cholesterol in spectroscopic-grade chloroform. After the chloroform was evaporated under a stream of nitrogen gas, the samples were prepared at the following molar fractions of cholesterol: 0, 0.01, 0.02, 0.03, 0.04 and 0.05. They were hydrated with distilled water so that the concentration might be 10% w/w and then incubated at about 55°C for 2 h.

The precise heat capacity measurements were performed, using an ac calorimeter. Recently a light-radiation type ac microcalorimeter has been developed to measure the heat capacity of a small quantity of liquid with a high accuracy [8]. This was applied to the heat capacity measurements of the present samples. The heat capacity of the samples was determined within an accuracy of 1% in units of $Jcm^{-3}K^{-1}$. The measuring frequency was 0.6 Hz, i.e., the observed heat capacity is a dynamic one. To obtain the specific heat of the samples in units of $J \cdot g^{-1} \cdot K^{-1}$ the density of them is necessary. In the present study, we will deal with the heat capacity in units of $J \cdot cm^{-3} \cdot K^{-1}$. When the specific heat is required, it could be calculated from the temperature dependence of the specific volume for the same systems, which have been measured for instance by Melchior *et al.* [9].

Results and discussion

Figures 1 and 2 show the temperature dependence of the heat capacities for 0, 1, 2, 3, 4 and 5 mol% cholesterol in heating and cooling, respectively. Before starting heating run, the samples were kept at 23°C for half a day. The temperature scanning rate in these measurements was 0.04 deg/min both in heating and in cooling. In Figs 1 and 2 the plots were moved in the vertical direction so as not to lie one upon another. In this study, we are mainly interested in the traces of the anomalous heat capacities and therefore, it is only denoted that the bar in ordinate corresponds to 1 $J \cdot cm^{-3} \cdot K^{-1}$.

The results for pure DPPC exhibit a considerable temperature hysteresis between heating and cooling as shown in A in Figs 1 and 2. This sort of behaviour can be measured only by an ac calorimetric method, since this method is a steady-state method and then, the temperature can be scanned in both heating and cool-

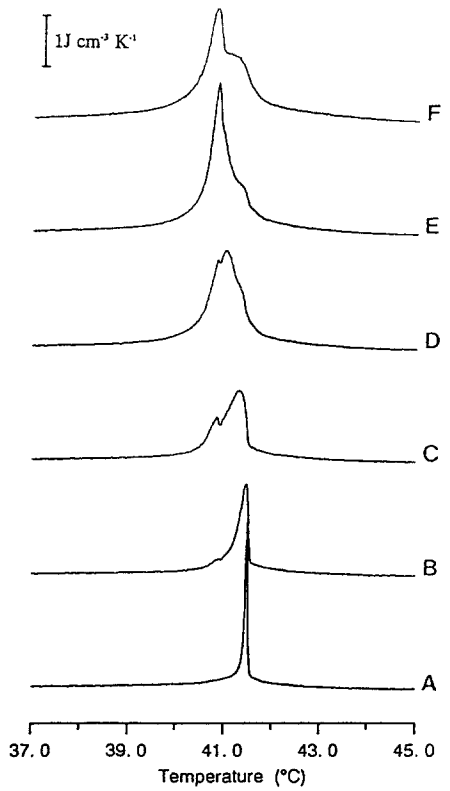

Fig. 1 Heat capacities of the dipalmitoylphosphatidylcholine-cholesterol systems in heating scan near the main transition. The cholesterol concentration is 0 mol% for A, 1 mol% for B, 2 mol% for C, 3 mol% for D, 4 mol% for E and 5 mol% for F

ing direction at any scan rate. The results are consistent with those obtained in the former experiments [10]. However, the results obtained by Tenchov *et al.* [10] and by us are in contradiction here to those previously obtained Imaizumi and Hatta [4]. This might be due to the fact that in the latter experiment a contamination with some impurities might not be excluded, since the surface of the suspension was sealed by silicone oil and the oil would interact with hydrophobic part of a phospholipid molecule. As expected from Figs 1 and 2 and seen in the former results [4], the temperature hysteresis is no longer visible above 8 mol% cholesterol.

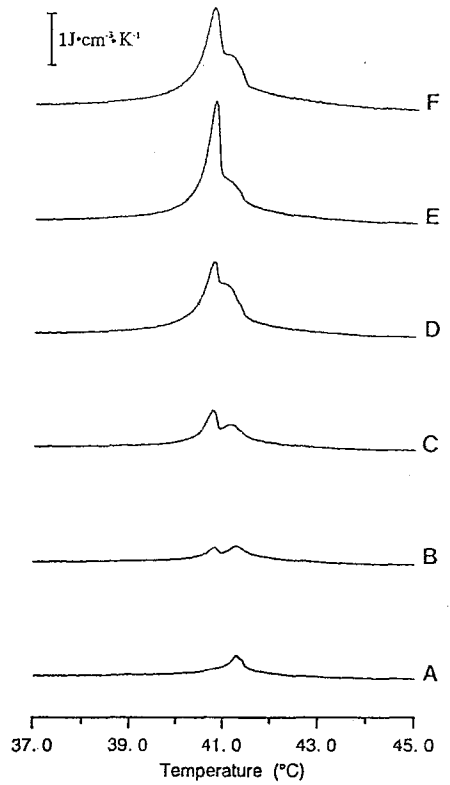

Fig. 2 Heat capacities of the dipalmitoylphosphatidylcholine-cholesterol systems in cooling scan near the main transition. The notations are the same as those of Fig. 1

The area of the anomalous part of the heat capacities was easily calculated by taking the flat straight line connecting the heat capacities at 37° and 45°C as the base line. Thus obtained dynamic enthalpies are shown in heating and cooling as a function of cholesterol concentration in Fig. 3. Then the dynamic enthalpies increase and the differences of the enthalpies between heating and cooling become

smaller with adding cholesterol. To show clearly the latter the ratio of the enthalpy in cooling to that in heating is plotted in Fig. 4 as a function of mol% cholesterol. As expected from this figure, the hysteresis of the dynamic enthalpies becomes smaller with increasing the cholesterol concentration. This quantitative result is consistent with the traces of Figs 1 and 2.

So far it has been pointed out in pure DPPC that the temperature hysteresis is due to the appearance of metastable ripple phase which takes place in cooling scan [10]. Furthermore, it has been revealed in the metastable ripple phase that in addition to the primary ripple structure the secondary ripple structure with repeat spacing a little smaller than twice repeat spacing in the primary ripple structure appears in part in cooling scan [11]. When the sample is cooled down to lower temperatures than the pretransition temperature and is heated up to the ripple phase, only the primary ripple structure takes place [10, 11]. With increasing the cholesterol concentration the hysteresis becomes smaller and then, the appearance of the secondary ripple structure is suppressed. It is worth pointing out that the above facts are consistent with the findings from the freeze-fracture microscopy of the phospholipid-cholesterol systems [6], in which the secondary ripple structure has not been observed above higher cholesterol concentrations than 5 mol%.

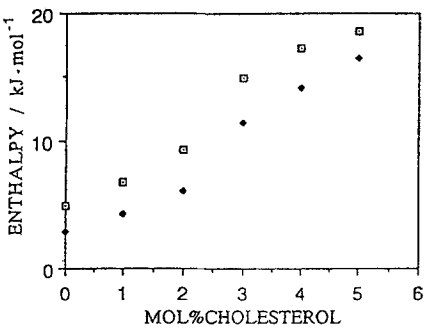

Fig. 3 Dependence of dynamic enthalpies on the cholesterol concentration in the dipalmitoylphosphatidylcholine-cholesterol systems in heating (denoted by □) and in cooling (denoted by ♦)

As discussed above, when we consider the ripple-to-liquid-crystalline phase transition, the results obtained in heating scan starting from temperature below the pretransition temperature should be dealt with and this is also the case in the study of the phase transition in the systems including the low cholesterol concentrations. Let us consider the detailed behaviour of the heat capacities. From the above reason, we focus our attention to Fig. 1. The curve F in Fig. 1 looks to be consistent with the results obtained before by differential scanning calorimetry [1, 2] and also by ac calorimetry [4]. However, it should be noted that at the

cholesterol concentrations lower than 5 mol% multipeaks appear in the heat capacity near the main transition. This fact suggests that in this temperature range a coexistence of low- and high-temperature phases which is derived from a simple theory for phase diagram does not take place, since in such phase diagram only two peaks of heat capacity should be observable. In pure DPPC denoted by A in Fig. 1, the heat capacity exhibits only a sharp peak at the main transition of 41.5°C. By the way in the trace of the heat capacity obtained by ac calorimetry we could not find anomaly at the pretransition of about 35°C. This is due to slow relaxation process at the pretransition [12] and therefore, the dynamic heat capacity measurements at 0.6 Hz do not observe anomalous behaviour.

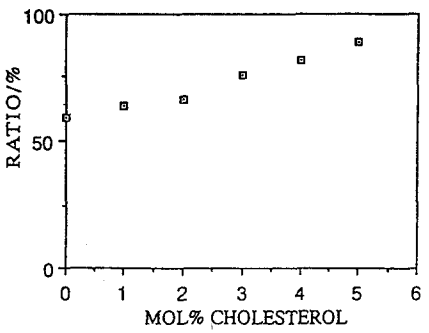

Fig. 4 Ratio of dynamic enthalpies in cooling to those in heating as a function of cholesterol concentration

In the system with 1 mol% cholesterol denoted by B, the contribution of the sharp peak at 41.5°C remains, but an additional anomalous part takes place in the low temperature side. Furthermore, a small peak grows at 40.8°C. In the system with 2 mol% cholesterol denoted by C, the contribution of the sharp peak at 41.5°C reduces and on the other hand, an anomaly at 41.3°C becomes pronounced. The peak at 40.8°C grows further and an additional shoulder appears at 41.0°C. In the system with 3 mol% cholesterol denoted by D, the contribution of the anomalies at 41.5° and 41.3°C remains still and the anomalies at 41.0° and 40.8°C become much more dominant. In the system with 4 mol% cholesterol denoted by E the anomaly at 40.8°C grows further and on the other hand the other anomalies reduce. In the system with 5 mol% cholesterol denoted by F, (a) the sharp anomaly at 40.8°C shifts to a little low temperature and becomes a little broad or (b) an additional peak takes place at 40.7°C. The latter, (b), might be the case, because we can find a small shoulder at 40.7°C in the traces of C, D and E. In the trace of F, the anomalies at 41.0°, 41.3° and 41.5°C are no longer distinguishable and form a unified shoulder.

It should be pointed out that the above appearance of the anomalies is not due to some particular reason which is caused by casual difference among the systems

at the various cholesterol concentrations, since the anomalies change systematically with increasing the cholesterol concentration.

It is a controversial point what concentration of cholesterol smears the heat capacity anomaly at the pretransition observed by differential scanning calorimetry. To make the point clear it is important to consider the relation between the ripple structure and the multipeak behaviour of heat capacity at the low cholesterol concentration. The concentration is as low as 3.6 mol% in the DSC experiment by Estep *et al.* [1]. The pretransition becomes not visible at cholesterol concentration above about 6 mol% in the DSC experiments by Mabrey *et al.* [2] and Vist and Davis [7]. In the experiments by Koynova *et al.* [5], the broad but clear anomaly remains up to 15 mol% cholesterol, but the transition temperature decreases more and more with the increase of the cholesterol concentration. We think that the results obtained by Koynova *et al.* manifest characteristic of this phase transition, because the results obtained with freeze-fracture electron microscopy indicate that the ripple structure takes place in the lower cholesterol concentration than 20 mol% [3]. Therefore, below 20 mol% cholesterol the systems undergo phase transitions from gel-to-ripple phase and from ripple-to-liquid-crystalline phase. Furthermore, as pointed out in this paper the latter phase transition is not a usual two state transition. We would point out that the nature of the ripple phase in the DPPC-cholesterol systems is not expressed in terms of so-called solid solution in a two component system, i.e., the structure in the DPPC-cholesterol systems is not composed of domains of the ripple structure for pure DPPC and the structure of pure gel phase, but the ripple structure itself changes with adding cholesterol to pure DPPC. To understand this phenomenon together with the appearance of the sharp and broad peaks of the heat capacity near the main transition, we have proposed a model of microscopic phase separation as discussed prior to Experiments [4]. To interpret the multipeak heat capacity near the main transition for the low cholesterol concentration, it is required to know additional information on the detailed structure for microscopic phase separation. We at least speculate that the multipeak behaviour should be related to the existence of the ripple structure in the DPPC-cholesterol systems, in which ripple repeat spacing increases as a function of cholesterol concentration.

In the dimyristoylphosphatidylcholine-cholesterol systems, the trace of the heat capacity is much more simple in comparison with the DPPC-cholesterol systems, i.e., only two peaks are visible at the low cholesterol concentration (manuscript in preparation by S. Matuoka, M. Okada, H. Yao and I. Hatta). This should be considered in connection with the ripple structure as well. It is important to understand the difference between both dipalmitoylphosphatidylcholine-cholesterol and dimyristoylphosphatidylcholine-cholesterol systems.

References

1 T. N. Estep, D. B. Mountcastle, R. L. Biltonen and T. E. Thompson, Biochemistry, 17 (1978) 1984.
2 S. Mabrey, P. L. Mates and J. M. Sturtevant, Biochemistry, 17 (1978) 2464.
3 B. R. Copeland and H. M. McConnell, Biochim. Biophys. Acta, 599 (1980) 95.
4 S. Imaizumi and I. Hatta, J. Phys. Soc. Jpn., 53 (1984) 4476.
5 R. D. Koynova, A. I. Boyanov and B. G. Tenchov, FEBS Letters, 187 (1985) 65.
6 A. Hicks, M. Dinks and M. A. Singer, Biochim. Biophys. Acta, 903 (1987) 177.
7 M. R. Vist and J. H. Davis, Biochemistry, 29 (1990) 451.
8 H. Yao and I. Hatta, Jpn. J. Appl. Phys., 27 (1988) L121.
9 D. L. Melchior, F. J. Scavitto and J. M. Steim, Biochemistry, 19 (1980) 4828.
10 B. G. Tenchev, H. Yao and I. Hatta, Biophys. J., 56 (1989) 757.
11 H. Yao, S. Matuoka, B. Tenchov and I. Hatta, Biophys. J., 59 (1991) 252.
12 K. Tsuchida, I. Hatta, S. Imaizumi, K. Ohki and Y. Nozawa, Biochim. Biophys. Acta, 812 (1985) 249.

Zusammenfassung — Mittels AC-Kalorimetrie wurde bei Cholesterol-Konzentrationen von weniger als 5 mol% das Verhalten der Wärmekapazität im System Dipalmitoylphosphatidylcholin-Cholesterol untersucht. Es wurde gezeigt, daß sich die Wärmekapazität in der Nähe der Hauptumwandlung aus mindestens vier Anomalien zusammensetzt, d.h. bei der Wärmekapazität kann ein Multipeak beobachtet werden. Diese Tatsache zeigt, daß eine einfache Theorie, welche die Koexistenz zweier Phasen in einem Zweikomponenten-System erklärt, für die Multipeakregion nicht geeignet ist. Weiterhin sollte bei Überlegungen zum thermodynamischen Verhalten von Systemen eine Beziehung zwischen der Multipeak-Wärmekapazität bzw. der Welligkeitsstruktur und der Cholesterol-Konzentration berücksichtigt werden.