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 dipalmitoyl-phosphatidylcholine-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
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