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On the phase diagram of an L-dipalmitoylphosphatidylcholine/cholesterol mixture

R.D. Koynova, A.I. Boyanov and B.G. Tenchov

Central Laboratory of Biophysics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

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1. INTRODUCTION

Due to the frequent occurrence of phosphatidylcholine (PC) and cholesterol (Chol) in cell membranes, the interaction between these two lipids has become a subject of great interest [1–15]. It is well known that, when added to phospholipid bilayers, Chol acts as a plasticizer causing fluidization of the solid states and solidification of the fluid states (reviews [1,2]). Although very intensely studied by many physical techniques, the lateral organization of mixed bilayers containing Chol and phospholipids still remains rather obscure. The investigations published up to now have made clear that Chol and PC (dipalmitoyl-PC or dimyristoyl-PC in particular) form a rather heterogeneous mixture especially at temperatures below the phospholipid phase transition. However, there is no consent on the size and pattern of the compositional domains in the plane of the membrane. For example, two different points of view oppose a phase separation in the gel phase into a phase of almost pure PC and a mixed PC-Chol phase at about 20–25 mol% Chol [3–5] against an ordered single-phase mixture of PC and Chol [6].

The temperature-composition phase diagrams of PC/Chol mixtures have been recorded by several different methods including the use of spin [4,7,10] and fluorescent [3,8,11] labels, freeze-fracture electron microscopy [3,4], microcalorimetry [9,12,13]. With all published data having been considered, a very detailed phase diagram of the mixture of dipalmitoylphosphatidylcholine (DPPC) with amounts of Chol up to about 50 mol% has been constructed [3]. This diagram contains a great number of different two-phase regions in the solid state of the mixture thus indicating a very complex picture of lateral interactions of these two lipids.

This paper reports differential scanning calorimetric (DSC) data on the following two facets of the PC/Chol phase diagram: (i) the behavior of the L-DPPC subtransition and pretransition with increase in Chol content; (ii) the mode of the disappearance of the main transition of DPPC with increase in Chol content. With respect to the latter point the DSC results suggest the existence of a critical point at 30–35 mol% Chol.

2. MATERIALS AND METHODS

Commercial grade L-DPPC (Fluka AG) and Chol (Merck) were used without further purification. By thin-layer and gas chromatography the phospholipid was shown to be of over 99% purity. The anhydrous Chol was checked by DSC and found to demonstrate the standard transition at 147°C.

Required amounts of stock solutions of L-DPPC and Chol in chloroform were mixed and the chloroform removed by rotary evaporation under

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nitrogen. The dry mixture was hydrated overnight in 50 mM Hepes, pH 7.2, at room temperature, then heated to 50°C for 1 h and shaken on a vortex mixer several times for about 30–40 s. The suspension was stored at 0–4°C for 4 days to induce the appearance of the subtransition [16].

DSC was performed with a Privalov differential adiabatic scanning calorimeter (DASM-1M) with a sensitivity better than 4×10^{-6} cal·K^{-1} and a noise level less than 5×10^{-7} W [17]. The DSC traces were recorded during heating of the samples. The heating rate was 0.5°C·min^{-1}. The onset and completion of the phase transitions were determined by the intersections of the peak slopes with the baseline on the thermograms [18].

Double glass-distilled water was used throughout all experiments. All chemicals were analytical grade.

3. RESULTS AND DISCUSSION

3.1. *L-DPPC subtransition and pretransition*

The calorimetric scans in fig.1 show the behavior of the L-DPPC subtransition and pretransition with increase in Chol mole fraction. The subtransition disappears at about 20 mol% Chol. Its decrease is accompanied by a slight shift to higher temperatures and also by an asymmetric broadening.

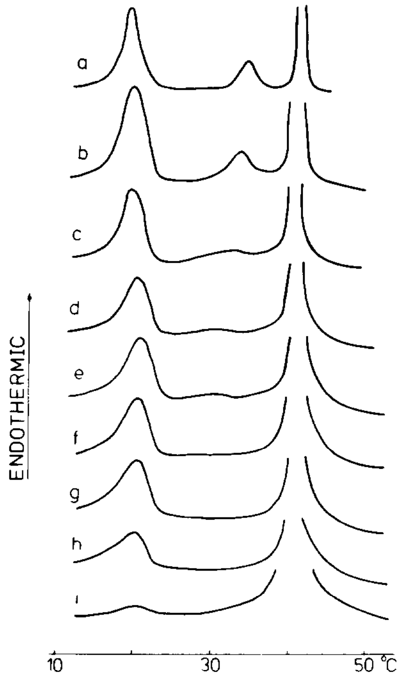

Fig.1. DSC scans of L-DPPC/Chol mixtures after 4 days storage at 0°C. Chol (mol%): (a) 0, (b) 3, (c) 5.7, (d) 8.4, (e) 11, (f) 13, (g) 15.5, (h) 19, (i) 23.

Upon addition of admixtures to DPPC bilayers, the temperature of the pretransition may either increase until its fusion with the main transition (e.g., dimyristoylphosphatidylcholine/dipalmitoylphosphatidylethanolamine or DPPC/palmitic acid mixtures [20,21]), or decrease [22]. It follows from our data that the DPPC-pretransition shifts towards the subtransition with increase of Chol but becomes unobservable before it merges with the subtransition (figs 1 and 3). This conclusion disagrees with the fluorescence and freeze-fracture data, which indicate an upward shift [3,8], but seems to be in accordance with the earlier DSC data (fig.1 in [12]).

3.2. *Mode of disappearance of the main transition*

It has been concluded on the basis of earlier DSC studies that the main transition of L-DPPC broadens until disappearance upon addition of increasing amounts of Chol [9,14]. However, it seems impossible to envisage a plausible physical model for such type of evolution of a solid-liquid first-order phase transition. In addition, as a result of the above conclusion, the positions of the solidus and liquidus curves in the binary temperature-composition phase diagram become perfectly indefinite at increasing amounts of Chol.

Fig.2 presents DSC scans of DPPC/Chol mixtures for mole fractions of Chol between 0.23 and 0.43. A careful examination of these scans (and a number of repetition scans not shown here) shows that the disappearance of the phase transition is not accompanied by an unlimited broadening of the transition, but rather by increase at first and then by decrease of the temperature interval between the transition onset and completion. In fact, this possibility does not seem to be inconsistent with the earlier DSC results. The conclusion about the unlimited broadening of the phase transition has been made on the basis of the measurement of the transition width at half-height [9,14]. However, as the slopes of the broad phase transition change from concave to convex with increase of Chol, the transition width at half-height may in-

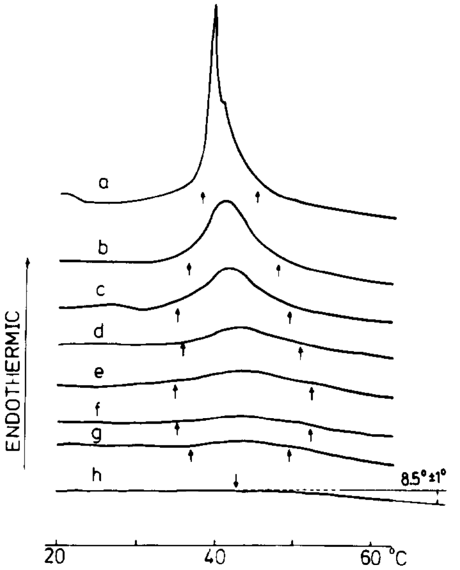

Fig.2. DSC scans of L-DPPC/Chol mixtures at higher Chol contents. Chol (mol%): (a) 23, (b) 28, (c) 31, (d) 35, (e) 38, (f) 41, (g) 43, (h) 53. The arrows indicate the intersections of the peak slopes with the baseline, used for construction of the phase diagram; the downward arrow at the last scan indicates the position of the baseline kink.

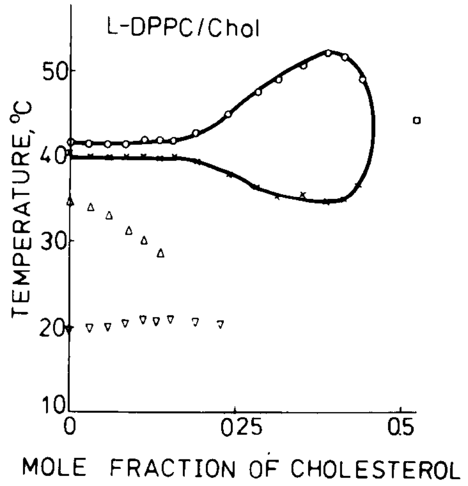

Fig.3. Temperature-composition phase diagram of L-DPPC/Chol mixture: (▽) midpoint of the subtransition, (△) midpoint of the pretransition, (×) onset of the main transition, (○) completion of the main transition, (□) position of the baseline kink after disappearance of the main transition.

crease in the absence of an increase in the temperature interval between transition onset and completion. This point is illustrated by the several final scans in fig.2 which may be compared with fig.2 in [9] and fig.1 in [15] for additional confirmation. It is important to note that the procedure adopted here for determining the transition onset and completion although commonly used [18] is, in fact, arbitrary and cannot be justified theoretically. It is clear that some other choice may change the position of the solidus and liquidus phase lines in fig.3 but the general conclusion about the mode of disappearance of the phase transition would nevertheless remain unaffected.

The alternative possibility proposed above suggests the existence of an immiscibility loop in the phase diagram (fig.3). Loops of this kind have been observed in liquid-liquid mixtures. These are related to the phenomenon of retrograde condensation [23]. As explained in [23], loops of this kind must be due to the existence of a critical point (at 30–35 mol% Chol in the present case). This alternative explanation has the important advantage of reconciling the disappearance of the phase transition with a realistic phase diagram. However, it must be kept in mind that, due to the very small magnitudes of the transitions which become comparable to the baseline deviations, this explanation should be considered as hypothetical. It cannot be definitely proved even by high-sensitivity DSC and must be checked by other methods. For this reason, no attempts were made to fit the diagram in fig.3 to the rules for construction of phase diagrams [24].

An additional noteworthy observation concerns the properties of the baseline. At variance with the baseline for a pure L-DPPC bilayer which is strictly horizontal [16,19], the baseline in the mixture exhibits a kink pointing upwards and located at approx. 42°C which persists on the thermograms after the disappearance of the phase transition (fig.2h). It may be assumed that a kink in the heat capacity curve reflects a phase transition of a higher order which replaces the first-order L-DPPC transition at higher Chol contents.

REFERENCES

[1] Oldfield, E. and Chapman, D. (1972) FEBS Lett. 23, 285–297.

[2] Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132.

[3] Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1943–1954.

[4] Kleemann, W. and McConnell, H. (1976) Biochim. Biophys. Acta 419, 206–222.

[5] Copeland, B.R. and McConnell, H. (1980) Biochim. Biophys. Acta 599, 95–109.

[6] Hui, S.W. and He, N.-B. (1983) Biochemistry 22, 1159–1164.

[7] Shimshick, E.J. and McConnell, H. (1973) Biochem. Biophys. Res. Commun. 53, 446–451.

[8] Lee, A.G. (1976) FEBS Lett. 62, 359–363.

[9] Mabrey, S., Mateo, P.L. and Sturtevant, J. (1978) Biochemistry 17, 2464–2468.

[10] Recktenwald, D.J. and McConnell, H. (1981) Biochemistry 20, 4505–4510.

[11] Rubenstein, J.L.R., Smith, B.A. and McConnell, H. (1979) Proc. Natl. Acad. Sci. USA 76, 15–18.

[12] Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340.

[13] Ter-Minassian-Saraga, L. and Madelmont, G. (1982) FEBS Lett. 137, 137–140.

[14] Hinz, H.-J. and Sturtevant, J. (1972) J. Biol. Chem. 247, 3697–3700.

[15] Estep, T.N., Mountcastle, D.B., Biltonen, R.L. and Thompson, T.E. (1978) Biochemistry 17, 1984–1989.

[16] Chen, S.C., Sturtevant, J.M. and Gaffney, B.J. (1983) Proc. Natl. Acad. Sci. USA 77, 5060–5063.

[17] Privalov, P.L., Plotnikov, V.V. and Filimonov, V.V. (1975) J. Chem. Thermodyn. 7, 41–47.

[18] Mabrey, S. and Sturtevant, J. (1976) Proc. Natl. Acad. Sci. USA 73, 3862–3866.

[19] Boyanov, A.I., Tenchov, B.G., Koynova, R.D. and Koumanov, K.S. (1983) Biochim. Biophys. Acta 732, 711–713.

[20] Luna, E. and McConnell, H. (1978) Biochim. Biophys. Acta 509, 462–473.

[21] Schullery, S.E., Seder, T.A., Weinstein, D.A. and Bryant, D.A. (1981) Biochemistry 20, 6818–6824.

[22] Boyanov, A.I., Koumanov, K.S., Karanov, E.H. and Barth, A. (1983) CR Acad. Bulg. Sci. 36, 811–814.

[23] Landau, L.D. and Lifshitz, E.M. (1959) Statistical Physics, Pergamon, London.

[24] Gordon, P. (1968) Principles of Phase Diagrams in Material Systems, McGraw-Hill, New York.