Biochimica et Biophysica Acta, 688 (1982) 23–28
Elsevier Biomedical Press

BBA 71128

PHOSPHOLIPID MISCIBILITY IN TERNARY MIXTURES

STEFANO MASSARI

C.N.R. Unit for the Study of Physiology of Mitochondria, Laboratory of Biophysics and Molecular Biology, Institute of General Pathology, University of Padua (Italy)

(Received November 27th, 1981)

Key words: Ternary mixture; Phospholipid; Miscibility; Lateral phase separation; Phase diagram

The gel to liquid-crystalline phase transition of aqueous dispersions of phospholipid mixtures was investigated by means of the repartition of the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl between aqueous space and lipid hydrocarbon region. The dimyristoylphosphatidylcholine (DMPC)/dibehenoylphosphatidylcholine (DBPC) and dipalmitoylphosphatidylcholine (DPPC)/DBPC phase diagrams indicate gel phase immiscibility, whereas the distearoylphosphatidylcholine (DSPC)/DBPC phase diagram indicates non-ideal gel phase miscibility at low DBPC molar fractions. Aqueous dispersions of DMPC/DPPC/DBPC ternary mixtures show two distinct phase transitions, the first associated with the melting of a DMPC/DPPC phase and the second with the melting of a DBPC phase. Aqueous dispersions of DMPC/DSPC/DBPC ternary mixtures show to phase transitions at low DSPC molar fractions; the first is probably associated with the melting of a DMPC/DSPC phase, and the second with the melting of a DBPC/DSPC phase. At high DSPC molar fractions, only one phase transition is observed; this suggests that all the lipids are mixed in gel state membranes.

Introduction

Natural membrane lipids generally consist of many components, including cholesterol and several kinds of phospholipid with varying acyl chain lengths and degrees of saturation. Because of this heterogeneity, non-ideal miscibility of phospholipids may be expected. Moreover, the presence of lipid clusters either in liquid-crystalline state membranes [1] or in the wide range of the lipid transition temperatures, the presence of proteins and external agents, such as $CaCl_2$, may greatly affect the phospholipid miscibility. The miscibility properties of the membrane phospholipids could play an important role in modulating enzyme activity, in reorganizing membrane components and in regulating ion permeability, as well as membrane aggregation and fusion.

Many attempts have therefore been made to clarify the nature of phospholipid mixing and its molecular mechanism, by using simple systems consisting of aqueous dispersions of phospholipid binary mixtures. Many techniques have been applied: ESR [2–4], calorimetry [5–7], electron microscopy [4,8], Raman spectroscopy [9], X-ray and electron diffraction spectroscopy [10], dilatometry [11], fluorescence [12] and ^{19}F-, ^{13}C- and ^{1}H-NMR [13–15].

With a view to approximating further a heterogeneous mixture of lipids in biomembranes, we studied mixed membranes containing three phospholipids. The phospholipids used were saturated

Abbreviations: TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DBPC, dibehenoylphosphatidylcholine.

diacyl phosphatidylcholines with 14 (DMPC), 16 (DPPC), 18 (DSPC) and 22 (DBPC) carbon atoms in the acyl chain. The results indicate that if a third lipid is added to a gel state membrane consisting of two immiscible lipids, it is possible to mix all the lipids only when the third component is miscible with the two single lipids in gel state membranes, and its concentration in the membrane is relatively high.

Materials and Methods

The purity of all the phospholipids, supplied by Sigma, was assayed by thin-layer chromatography: all showed single spots. Multilayered vesicles were prepared by drying in vacuo a chloroform solution of phospholipids (30 μmol). Traces of the solvent were removed by adding 100 μl light petroleum ether. 1 ml of an aqueous solution containing 300 μM TEMPO, 0.1 M KCl and 10 mM Tris-HCl (pH 7) was added above the higher lipid transition temperature. The flask was flushed with nitrogen and shaken until all lipids adhering to the bottom were dispersed. The phospholipid dispersion was slowly cooled and maintained at a temperature of 10°C for 24 h before use.

EPR measurements were obtained with a Varian E-12 spectrometer at X band. The temperature was controlled to within ± 1°C in the 10–80°C range and measured with a thermocouple immersed in the center of the pipet used as the sample cell. The spectra were obtained as a function of the temperature with a heating rate of no more than 10 C deg./h. At temperatures ranging from 50 to 60°C, the lipid slowly aggregates and separates from the aqueous medium. This phenomenon persists even when different buffers in the sample or degassed solutions are used, and seems to be present only when thin capillary tubes are employed. The lipid suspension therefore was mechanically stirred at these temperatures.

Results and Discussion

The TEMPO paramagnetic resonance spectra in lipid aqueous dispersions are a superposition of the spectrum of the label in the aqueous phase and the spectrum of the label dissolved in the liquid hydrocarbon region. The TEMPO spectral parameter, f, calculated according to Shimshick and McConnell's method [2], is approximately the fraction of spin label dissolved in the membrane bilayer, and exhibits abrupt changes in the proximity of the temperatures corresponding to the calorimetrically measured gel to liquid-crystalline transition temperatures.

Fig. 1 shows the dependence of f on the temperature for aqueous dispersions of DMPC/DBPC, DPPC/DBPC and DSPC/DBPC binary mixtures, at two DBPC molar fractions. Two distinct phase transitions are detected in DMPC/DBPC and DPPC/DBPC mixtures. The low-temperature transition is the same as that of DMPC and DPPC vesicles, respectively, and no temperature shift is observed when the DBPC molar fraction is changed. This indicates immiscibility between DMPC or DPPC and DBPC in gel phase membranes. An alternative explanation is that DMPC/DBPC and DPPC/DBPC aqueous dispersions do not form mixed bilayers, but rather distinct bilayers, each being formed by the single component. In this case, however, the high temperature transition should be the same as that of DBPC vesicles and, contrary to the results shown in Fig. 1A and B, it should show no temperature shift on changing the DBPC molar fraction. The spectral parameter curves of DSPC/DBPC binary mixtures are more complex (Fig. 1C) since their shape is biphasic up to 0.5 DBPC molar fractions, and monophasic at higher DBPC molar fractions. Similar behavior has been observed for lipid mixtures in which the shape of the phase diagram suggested gel phase non-ideal miscibility [2,12].

Fig. 2 shows the gel to liquid-crystalline phase diagrams for aqueous dispersion of DMPC/DBPC, DPPC/DBPC and DSPC/DBPC mixtures. The experimental temperatures were measured at the first inflection point of the low-temperature transition curve (T_1, Fig. 1) and at the last inflection point of the high-temperature transition curve (T_1', Fig. 1). The phase diagrams shown in Fig. 2A and B indicate that DMPC and DPPC do not mix with DBPC in gel phase membranes. This finding confirms that when the disparity in chain length of two phosphatidylcholines is equal to or more than six carbon atoms, such as in dilauroylphosphatidylcholine/distearoylphosphatidylcholine and in dimyristoylphosphatidylcholine/dielaidoylphos-

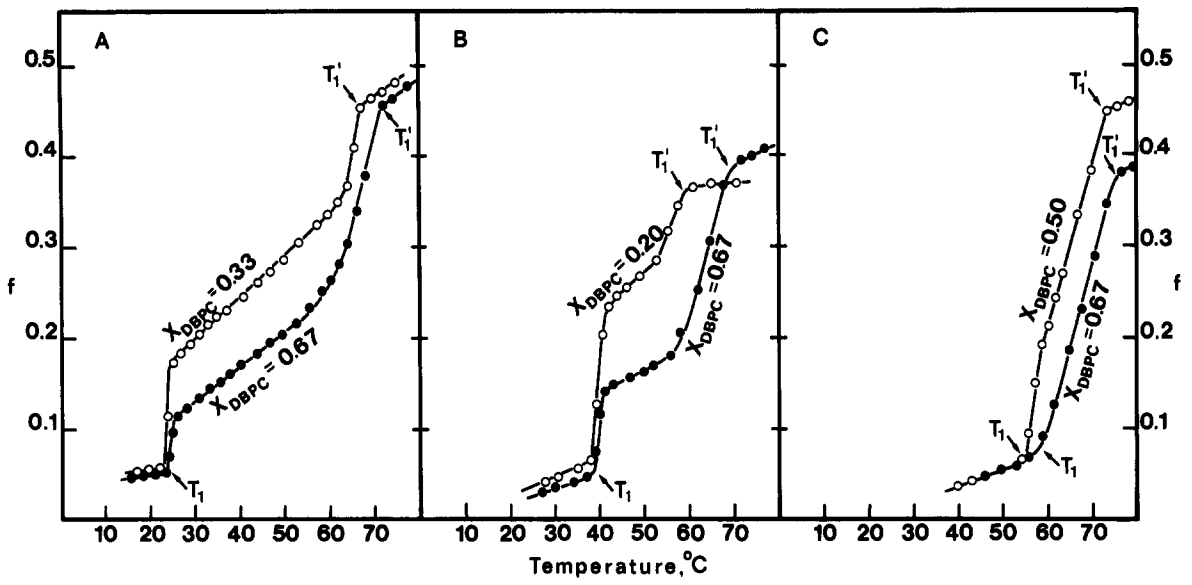

Fig. 1. The TEMPO spectral parameter, f, as a function of the temperature for aqueous dispersions of: (A) DMPC/DBPC, (B) DPPC/DBPC and (C) DSPC/DBPC. The DBPC molar fractions are indicated.

phatidylcholine mixtures, there is immiscibility of the two components in the gel state [6,11]. The phase diagram of DSPC/DBPC mixtures (Fig. 2C) shows a solidus curve close to a horizontal line at low DBPC molar fractions. In this respect, the DSPC/DBPC system behaves like DMPC/DSPC system, in which the two lipids also differ four carbon atoms in chain length and for which non-

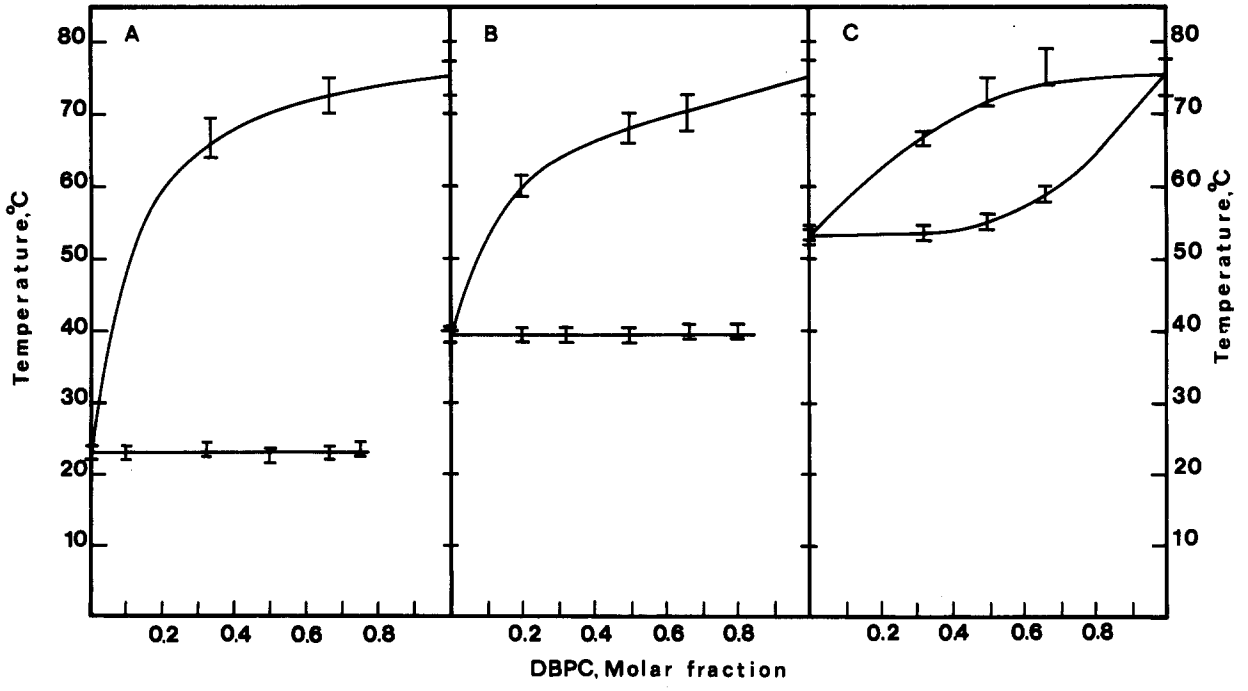

Fig. 2. The gel to liquid-crystalline equilibrium phase diagrams for aqueous dispersions of: (A) DMPC/DBPC, (B) DPPC/DBPC and (C) DSPC/DBPC.

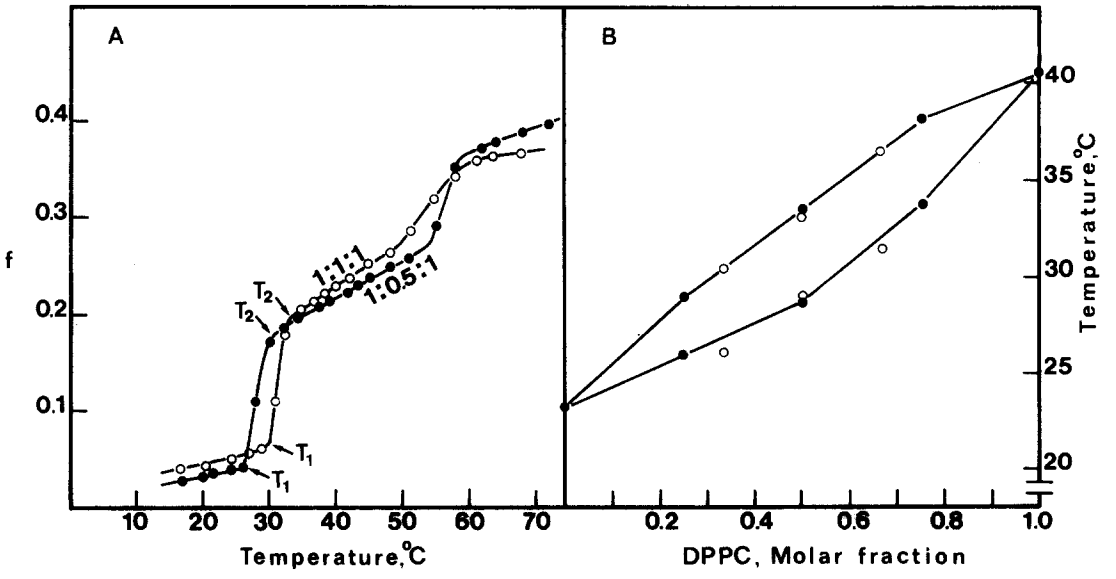

Fig. 3. (A) The TEMPO spectral parameter as a function of the temperature for DMPC/DPPC/DBPC aqueous dispersions. The relative ratios of the lipids are shown. (B) The gel to liquid-crystalline phase diagram for aqueous dispersion of DMPC/DPPC. ●, experimental data obtained from Shimshick and McConnell experiments [2]. ○, experimental temperatures obtained from the TEMPO spectral parameter curves of Fig. 3A (T_1 and T_2). The DPPC molar fractions in the ternary mixtures were calculated as: DPPC/(DMPC+DPPC). The DMPC/DBPC ratio was 1 : 1 in all the ternary mixtures used.

ideal lipid miscibility at low DSPC molar fractions has been suggested in the gel state [2].

Fig. 3A shows the TEMPO spectral parameter as a function of the temperature for aqueous dispersions of DMPC/DPPC/DBPC ternary mixtures. Two distinct phase transitions are observed, indicating that two phases of different lipid composition exist in the gel state membrane. The low-temperature transition, characterized by the temperatures T_1 and T_2 corresponding to the two inflection points of the transition curve (Fig. 3A), is shifted to higher temperatures when the DPPC molar fraction is increased. This shift may be due either to a progressive enrichment of DPPC in a DMPC phase, or to a progressive solubilization of DBPC in a DMPC/DPPC phase. In the former case, T_1 and T_2 should be equal to or lower than the two temperatures calculated on the solidus and liquidus lines of the phase diagram of DMPC/DPPC binary mixtures, at the same DPPC molar fractions *: they would be equal when all DPPC in the ternary mixture has been solubilized in the DMPC phase, and lower when part of DPPC has been solubilized in the DBPC phase. In the latter case T_1 and T_2 should be higher than the corresponding temperatures calculated in the DMPC/DPPC phase diagram. In Fig. 3B T_1 and T_2 are compared with the temperatures reported by Shimshick and McConnell [2] in the phase diagram of DMPC/DPPC aqueous dispersions. The close fitting of T_1 and T_2 with the phase diagram of the binary mixture suggests that the phases present in DMPC/DPPC/DBPC membranes in the gel state consist of DMPC/DPPC and the single DBPC component. This result is in agreement with the observation that DPPC, which is completely soluble in DMPC membranes in the gel state, is immiscible with DBPC membranes in the gel state (Fig. 2).

Fig. 4 shows the TEMPO spectral parameter as a function of the temperature for aqueous dispersions of DMPC/DSPC/DBPC ternary mixtures. The presence of two distinct transitions at low DSPC molar fractions and of only one at higher DSPC molar fractions indicates that by increasing

* The DPPC molar fraction in the ternary mixtures is calculated as: DPPC/(DMPC+DPPC).

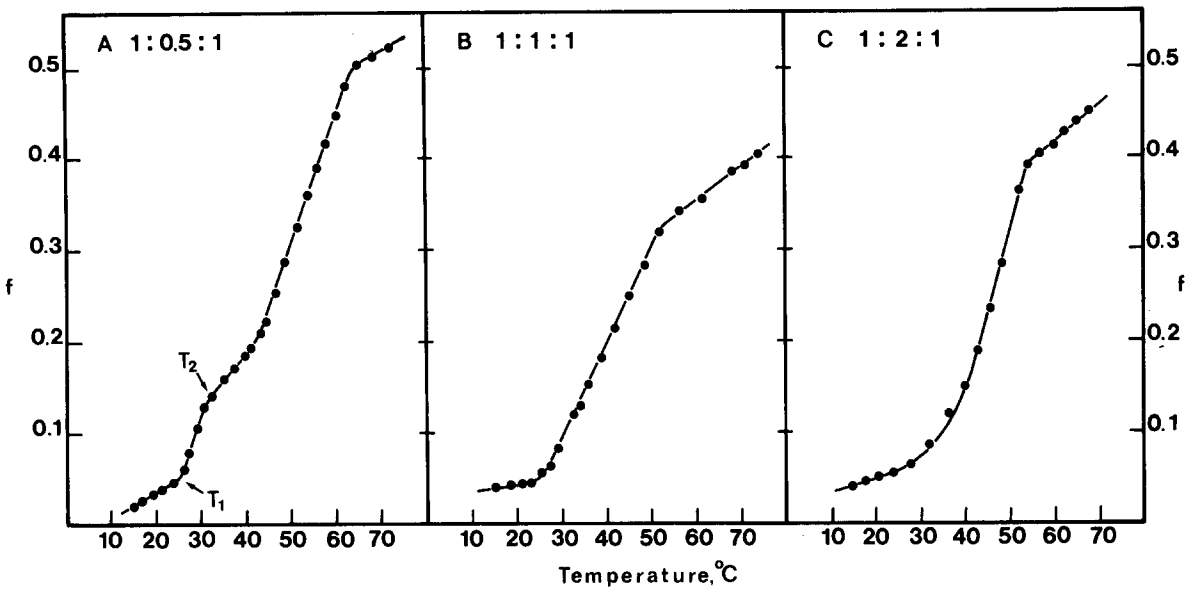

Fig. 4. The TEMPO spectral parameter as a function of the temperature for DMPC/DSPC/DBPC aqueous dispersions. The relative ratio of the lipids are shown.

the amounts of DSPC, the miscibility of all the lipids present in the system is progressively enhanced. The low temperature transition shown in Fig. 4A would correspond to the melting of a DMPC phase enriched with DSPC and perhaps, with DBPC. It is still not known, however, how much DSPC and DBPC are present in this phase. The two temperatures, T_1 and T_2, at which this phase initiates and terminates the melting process are 25.5 and 32°C respectively. If DBPC is absent and all DSPC is present in this phase, T_1 and T_2 should be equal to the two temperatures calculated on the solidus and the liquidus lines of the phase diagram of DMPC/DSPC binary mixtures at the same DSPC molar fraction *, which are approx. 26 and 40°C, respectively [2]. The lower temperature range found in the ternary mixture suggests that only part of DSPC is localized in the DMPC phase, and that DBPC is present only in trace amounts. Since 25.5–32°C in the DMPC/DSPC phase diagram correspond approximately to a mixture of DMPC and 30% of DSPC, we may conclude that the first transition of the TEMPO spectral curve of Fig. 4A corresponds to the melting of a DMPC phase enriched with approx. 30% of DSPC and, perhaps, traces of DBPC, whereas the second transition corresponds to the melting of a DBPC phase enriched with approx. 70% of DSPC and, perhaps, traces of DMPC. This conclusion confirms that lipids (in our case DSPC) are more soluble in a phase consisting in longer chain-length lipids (DBPC) than in a phase consisting in shorter chain-length lipids (DMPC) in gel state membranes [5].

* The DSPC molar fraction in the ternary mixture in calculated as: DSPC/(DMPC+DSPC).

Acknowledgments

The author would like to thank Mr. B. Ziche for his technical assistance and Dr. R. Colonna for helpful discussion.

References

1 Lee, A.G., Birdsall, N.J.M., Metcalfe, J.C., Toou, P.A. and Warren, G.B. (1974) Biochemistry 18, 3699–3705
2 Shimshick, E.J. and McConnell, H.M. (1973) Biochemistry 12, 2351–2360

3 Wu, S.H. and McConnell, H.M. (1975) Biochemistry 14, 847–854
4 Luna, E.J. and McConnell, H.M. (1977) Biochim. Biophys. Acta 470, 303–316
5 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152–161
6 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 3862–3866
7 Van Dijck, P.W.M., Karper, A.J., Oonk, H.A.J. and De Gier, J. (1977) Biochim. Biophys. Acta 470, 58–69
8 Hui, S.W. (1981) Biophys. J. 34, 383–395
9 Mendelsohn, R. and Maisano, J. (1978) Biochim. Biophys. Acta 506, 192–201
10 Stewart, T.P., Hui, S.W., Portis, A.R. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 556, 1–16
11 Wilkinson, D.A. and Nagle, J.F. (1979) Biochemistry 18, 4244–4249
12 Lentz, B.R., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4529–4537
13 Gent, M.P.N. and Ho, C. (1978) Biochemistry 17, 3023–3038
14 Jacobs, R. and Oldfield, E. (1979) Biochemistry 18, 3280–3285
15 Van Echteld, C.J.A., De Kruijff, B. and De Gier, J. (1980) Biochim. Biophys. Acta 595, 71–81