Studying the phase behavior of lipids and binary lipid mixtures by differential scanning calorimetry

Lipids are an important component in biological membranes. One of the most common classes of membrane lipids is phospholipids, which play a key role in both the structure and function of cellular membranes. Two members of this class are 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Fig. 1). These are amphiphilic lipids with very low solubility in water (in the order of 10^{-9} M).

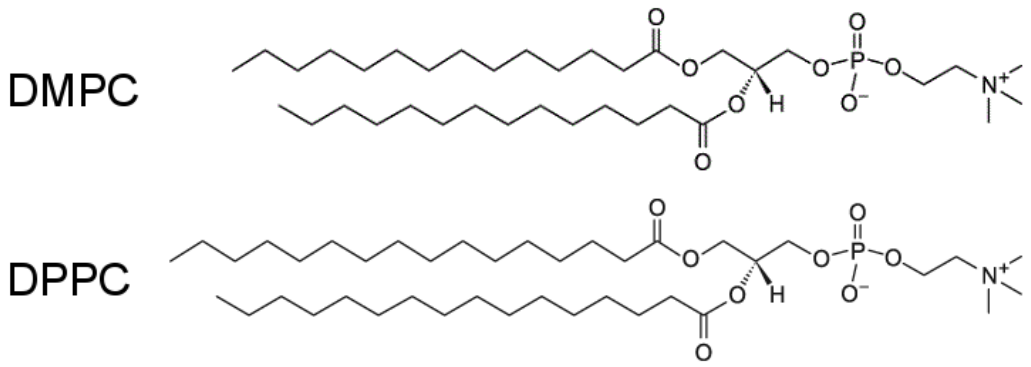

Fig. 1. Chemical structure of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC).

Typically, phospholipids self-assemble into bilayer stacks with more or less order depending on water and temperature conditions. In excess water they can form multilamellar vesicles, which constitute a dispersed lamellar phase (Fig. 2).

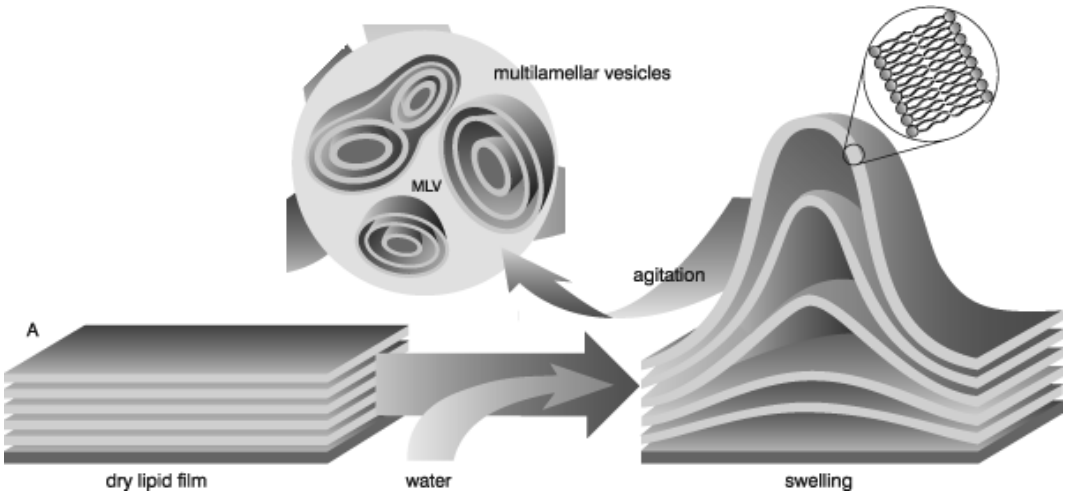

Fig. 2. Formation of multilamellar vesicles [1], which are used in this lab.

As can be seen in Fig. 3, when dispersed in water these lipids display thermotropic (temperature induced, vertical direction in Fig. 3a) and lyotropic (water content induced, horizontal direction in Fig. 3a) phase transitions. Thermotropic phase transitions in water-lipid systems are often detected by differential scanning calorimetry (DSC). A DSC instrument contains two cells that are thermally

insulated from the surroundings. One of the cells contains the sample and the other one contains a reference (typically the buffer solution of the sample). In a DSC experiment, the temperature of the sample cell and the reference cell is increased or decreased at a constant rate (°C/s). The temperature is always kept equal between the two cells. In order to do that the power used to heat or cool each cell will be continuously adjusted to counteract any difference between them. The difference in power (J/s) applied to the sample cell compared to the reference cell is measured and can then be converted to a difference in heat capacity (J/°C) between the sample and the reference by scan rate normalization. A thermally induced phase transition results in a positive or negative peak on the DSC trace, depending on whether the transition is endothermic or exothermic [3]. The corresponding molar enthalpy change (J/mol) for the transition can be calculated from the areas under the peaks, together with knowledge of the concentrations and volumes.

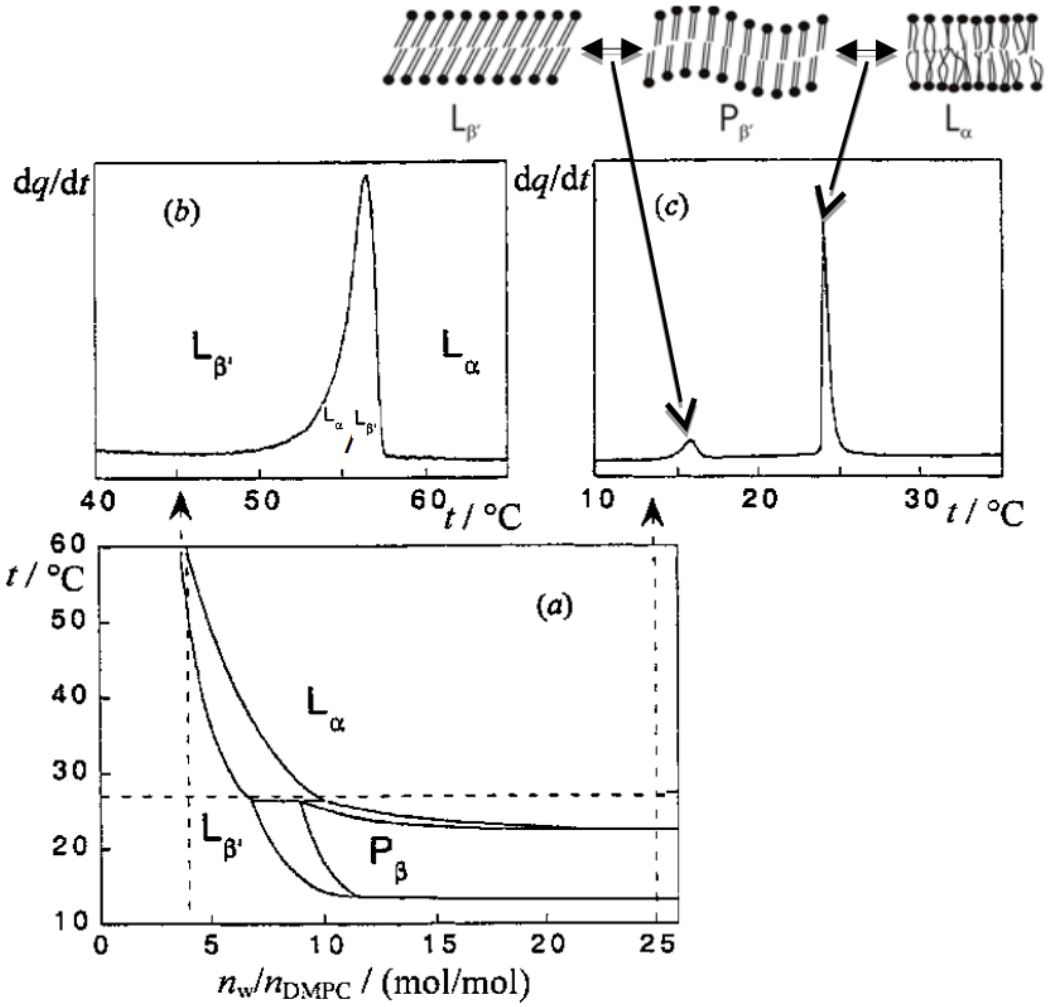

Fig. 3. (a) A theoretically calculated phase diagram of DMPC. (b) DSC trace of hydrated DMPC at low water content. (c) DSC trace of hydrated DMPC at high water content [2].

Fig. 3c shows a typical DSC thermogram of DMPC at high water content. Two different solid-like phases (called gel phases, $L_{\beta'}$ and $P_{\beta'}$) and a lamellar liquid crystalline phase, L_α,can be observed. In the gel phases, the hydrocarbon chains are crystalline and conformationally ordered. There are different gel phases due to different ways of packing the chains and the headgroups. During the so called pretransition (the small peak in Fig. 3c), there is a change from the $L_{\beta'}$ gel phase with planar geometry and tilted chains to the $P_{\beta'}$ gel phase with periodic rippled structure due to expansion of

the headgroups. At higher temperature (the chain melting temperature), there is a main transition from the $P_{\beta'}$ phase to the L_{α} phase in which the chains become conformationally disordered [2].

Phase transitions in dilute water-lipid mixtures are characterized by well-defined transition temperatures. The phase transition temperature of the lipid varies with the length of the hydrocarbon chains and with degree of saturation. Moreover, the phase transitions are affected by the presence of impurities and other lipids. The presence of an impurity broadens a phase transition by introducing a two-phase coexistence [5, page 522]. Among other things, biological membranes consist of a mixture of many different lipids. The phase of the mixed multi-component lipid system determines the rate of transport across the membrane and other vital properties for its biological function. DSC is a useful tool to construct phase diagrams (transition temperatures versus composition), for example of binary lipid mixtures in excess water. Fig. 4 shows such a phase diagram for mixtures of DMPC and DPPC.

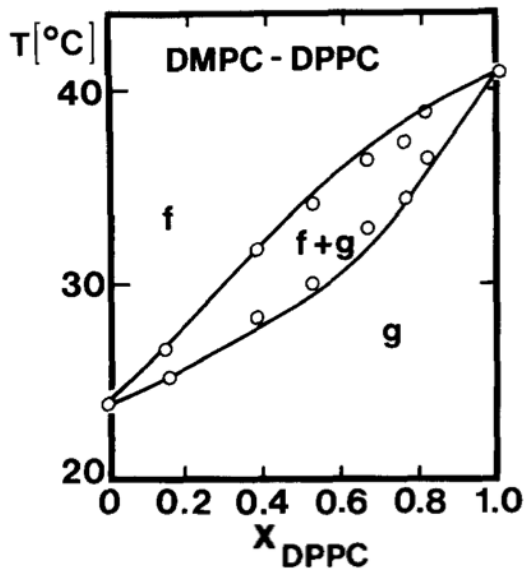

Fig. 4. Phase diagram for mixtures of DMPC and DPPC in excess water with theoretical values (solid lines) and experimental points (circles). The labels ("f" and "g") refer to the fluid (L_{α}) and the gel ($P_{\beta'}$ and $L_{\beta'}$) phases [4].

References

[1] Avanti Polar Lipids, Inc. “Avanti Polar Lipids, Inc”, Cited: August 30, 2013, http://avantilipids.com/index.php?option=com_content&view=article&id=1384&Itemid=372

[2] Markova, N., E. Sparr, et al. (2001). "On application of an isothermal sorption microcalorimeter." Thermochimica Acta 374(2): 93-104.

[3] MicroCal TM VP-Capillary DSC System - Getting Started Booklet. GE Healthcare Life Sciences.

[4] Mouritsen, O. G. (1991). "Theoretical models of phospholipid phase transitions." Chem Phys Lipids 57(2-3): 179-194.

[5] D. Fennell Evans, H. W. The Colloidal Domain: Where Physics, Chemistry, Biology, and Technology Meet WILEY-VCH.

Laboration

Two different phospholipids, DMPC and DPPC, are used in the experiment. The pure lipids and their mixtures are scanned in the DSC instrument from 10 °C to 60 °C with a scan rate of 60 °C/h. The concentrations of lipids in the samples are as follows:

Sample	DMPC (mg/ml)	DPPC (mg/ml)
1	2.00	0.00
2	1.37	0.63
3	0.96	1.04
4	0.57	1.43
5	0.38	1.62
6	0.00	2.00

The lipids are mixed and then dispersed in water to form multilamellar vesicles, which are a dispersed lamellar phase.

Because doing all the necessary DCS measurements would be very time consuming, each lab group will do measurements on only one sample, followed by analysis on a previous data set.

Before you come to the lab, please read

1. The lab instructions (this document).

2. The paper named “Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry” by Susan Mabrey and Julian M. Sturtevant (attached). Focus on the DMPC-DPPC system and the highlighted parts.

3. Chapter 6 “Bilayer Systems” in The Colloidal Domain book, which gives you knowledge about the lipid systems (already finished according to the schedule).

4. Part 10.3.3 and 10.3.4 (page 520, The Colloidal Domain book), which complement the paper.

There will be a small quiz before we start the lab. So make sure you know what the lab is about. You do not have to memorize any formulas, just understand the essentials.

In your report, you should include a brief introduction of the experiment, results and discussion, conclusions, and references. If you do not have these minimum requirements, your report will be sent back to you without being corrected and it will not be corrected until you have the minimum requirements. There is also a “guidelines” list that is provided to you. Please follow the guidelines and include the listed points in you report. Following the guidelines will help you to hand in a complete report. Make sure your name is included in the report.

Fig. 5. A photograph of the DSC instrument together with surrounding equipment at the division of physical chemistry at Lund university.

Guidelines for writing the DSC lab report

Points to include in the introduction part:

● Briefly describe the experimental setup, its main components and their functions. Explain accurately the working principles of the DSC.

● What is the relationship between the heat input to the cells in the absence of phase transitions? What happens to the DSC thermogram in the case of an endothermic phase transition? What if it is exothermic?

● Describe briefly how you perform a DSC scan. Why is it important to degas the samples? What could happen if you had an air bubble in one of the cells of during the DSC scan?

● How do you determine the transition temperature and calculate the enthalpy of a phase transition from the DSC scans?

Points to include in the results and discussion part:

● Plot the DSC thermograms of all samples in the same plot (zoom in to the transition region). Describe where you have different phases and phase coexistence in the plot.

● Make a table like the one below with all samples. Note: the peak areas from the instrument are in millicalories, so you need to make a conversion of units for the enthalpies. The temperatures should be the transition temperature for the pure lipids, and the transition temperature interval for the mixtures. Show detailed calculations for sample 4 as an example of how to calculate the values.

			Pretransition			**Main transition**		
Sample	**X_{DPPC}**	**$n_{total\ lipid}$ (µmol)**	**Area (mcal)**	**Tempera-ture (°C)**	**Enthalpy (kJ/mol)**	**Area (mcal)**	**Tempera-ture (°C)**	**Enthalpy (kJ/mol)**
...	**...**	**...**	**...**	**...**	**...**	**...**	**...**	**...**

● Explain a DSC thermogram. Locate the different phases and phase transitions. Explain the differences between the different phases. Discuss in terms of order and mobility of the lipids.

● Why is the main transition peak so much bigger than the pretransition peak? Relate to the events and transitions taking place, and how much energy they need.

● What do the DSC thermograms say about miscibility? Hint: for the mixtures, do we have one peak for each transition (pre- and main transition) or a superposition of the curves for the two pure compounds?

● Why do the two lipid species mix here, while that was not the case for the ones used in the AFM lab?

● How do the melting temperatures (pure compounds) or melting intervals (mixtures) and melting enthalpies vary with increasing mole fraction of DPPC? Explain in terms of intermolecular interactions. Comment critically on possible deviations from the expected behavior.

● Why do we have a melting interval and not a single melting temperature for the mixtures? Why do we have much thinner, sharper and higher peaks for the pure compounds? What should the main transition peak for the pure compounds ideally look like (for a first order phase transition)?

● Calculate an ideal mixing phase diagram (for the main transition). Present the equations, the values and the units you use and explain them. Use the transition temperatures and equations found in the paper by Susan Mabrey and Julian M. Sturtevant for your calculations. Show your calculation at T = 30 °C as an example.

● Plot in the same graph the experimental and the calculated phase diagram (for the main transition), with mole fraction of DPPC and temperature in degrees Celsius. Explain all the symbols, where you have the gel and fluid phases, and where you have their coexistence.

● Compare the calculated and the experimental phase diagrams. Why is the agreement good with such a simple model? Relate to the characteristics of the lipids. Even if the agreement is good, there are of course deviations. Where are these deviations more pronounced, why, and how could you correct for that?

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Biochemistry

Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry

(lipid bilayers/membranes/phase diagrams/transition heat capacity curves)

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Contributed by Julian M. Sturtevant, August 3, 1976

ABSTRACT **High sensitivity differential scanning calorimetry is applied to the study of the thermotropic behavior of mixtures of synthetic phospholipids in multilamellar aqueous suspensions. The systems dimyristoylphosphatidylcholine–dipalmitoylphosphatidylcholine, dimyristoylphosphatidylcholine–distearoylphosphatidylcholine, and dimyristoylphosphatidylethanolamine–distearoylphosphatidylcholine, although definitely nonideal, exhibit essentially complete miscibility in both gel and liquid crystalline states, while the system dilauroylphosphatidylcholine–distearoylphosphatidylcholine is monotectic with lateral phase separation in the gel state. Comparison of the observed transition curves with theoretical curves calculated from the calorimetrically determined phase diagrams supports a literal interpretation of the phase diagrams.**

The phase transitions of multilamellar suspensions of mixtures of synthetic phospholipids have been studied by low-sensitivity differential scanning calorimetry (1–5), by the spin label technique (6, 7), and by the use of chlorophyll *a* as a fluorescent probe (8). In view of the importance of lipid phase transitions and lateral phase separations (6, 7) in biological membranes, further study of synthetic systems by high-sensitivity differential scanning calorimetry (9) is warranted. In this paper we report on three binary systems that show essentially complete miscibility in both solid and liquid phases [dimyristoylphosphatidylcholine (DMPC)–dipalmitoylphosphatidylcholine (DPPC), DMPC–distearoylphosphatidylcholine (DSPC), and dimyristoylphosphatidylethanolamine (DMPE)–DSPC], and one that shows limited miscibility in the solid state [dilauroylphosphatidylcholine (DLPC)–DSPC].

The validity of phase diagrams, and of the Gibbs phase rule, for systems of the type considered here has been called into question by Lee (8) on the basis that the lipid systems are small systems in the thermodynamic sense. It appears to us, on the contrary, that the calorimetric evidence presented in this paper, together with the results of previous studies of pure phospholipids by high-sensitivity differential scanning calorimetry, indicates that these systems can be treated, in terms of the phase rule, in the same way as ordinary three-dimensional systems.

MATERIALS AND METHODS

DLPC, DMPC, and DPPC were obtained from Calbiochem and were used without further purification. DMPE and DSPC, obtained from the same source, were recrystallized five times from absolute ethanol and five times from absolute ethanol-acetone. The transition properties of both lipids were changed somewhat by purification. None of the lipids lost a significant amount of weight on heating at 50° in a vacuum oven for several hours, and they were thus assumed to be anhydrous. Our best current values for the transition properties of these lipids are given in Table 1, and differ significantly in some respects from the values previously reported from this laboratory (10, 11), presumably because of varying small amounts of impurities.

Lipid mixtures were prepared by dissolving the weighed components in a small volume of chloroform and removing the solvent at 40–50° in a vacuum oven. The appropriate amount of 0.01 M sodium phosphate buffer, pH 7.0, was added and the lipids were suspended by 2–3 min of shaking on a vortex mixer at about 60° under nitrogen. For mixtures containing DLPC it was necessary to use buffer containing 15% (vol/vol) of ethylene glycol to prevent freezing. The transition behavior of DSPC in this solvent was found to be very similar to that of DSPC in aqueous buffer, with just a slight broadening of the main transition. A small amount of settling observed with some of the mixtures seemed to have no effect on the calorimetric results.

All calorimetric scans were performed with the Privalov calorimeter (9), usually at 0.5° min^{-1}. Scan rates as low as 0.1° min^{-1} were occasionally used, and on the basis of these experiments we conclude that the systems were close to equilibrium throughout their phase transitions. With pure lipids, concentrations of 0.2–0.4 mg ml^{-1} were used; with mixtures, concentrations as high as 2–4 mg ml^{-1} were used. As would be expected with heterogeneous systems, there were no indications of any concentration dependence.

RESULTS AND DISCUSSION

Pure Lipids. The transitions of DLPC and DMPE, which have not previously been studied by high sensitivity differential scanning calorimetry, are shown in Fig. 1. It has been reported (12) that the latent heat of the transition of DLPC is 4.3 kcal mol^{-1}. The total area of the curve for DLPC in Fig. 1 from −4° to +10° corresponds to 4.77 kcal mol^{-1}. However the empirical correlation given in the insert in the figure, in which the point for C_{12}, 1.69 kcal mol^{-1}, is based on the area of the sharp peak only, gives strong support for taking this as the latent enthalpy for DLPC. The source of the heat absorption centered at about 4.5° is unknown; it is seen in aqueous suspension also, and disappears on addition of small amounts of lauric acid. The DLPC transition has the highest degree of cooperativity we have observed with phospholipids, with a cooperative unit (10) of approximately 1000 lipid molecules. Extrapolation of the line in the insert suggests that saturated lecithins with hydrocarbon chains shorter than 12 carbon atoms cannot form stable bilayers.

As is true of phosphatidylethanolamines in general, DMPE has only one transition, and this transition is definitely asymmetric.

Abbreviations: DLPC, dilauroylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine.

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Table 1. Transition properties of various phospholipids in multilamellar aqueous suspension

Lipid	Lower transition T_{m_1} (°C)	Lower transition ΔH_1 (kcal mol^{-1})	Lower transition Cooperative unit (molecules)*	Upper transition T_{m_2} (°C)	Upper transition ΔH_2 (kcal mol^{-1})	Upper transition Cooperative unit (molecules)*
DLPC	—	—	—	−1.8	1.7_0	980
DMPC	14.2	1.0_0	280	23.9	5.4_4	330
DPPC	35.3	1.8_3	290	41.4	8.7_4	260
DSPC	51.5	1.8_5	160	54.9	10.6_2	130
DMPE	—	—	—	49.5	5.8_0	140

* The size of the cooperative unit is particularly affected by impurities and other unrecognizable influences. The values given are maximum observed values.

DMPC-DPPC. The transition curves observed with two different mixtures of DMPC and DPPC are shown in Fig. 2A. The lower transitions appear at temperatures intermediate between those for the two components, but they have not been subjected to quantitative analysis. A significant asymmetry is apparent in the main transitions. In order to construct a phase diagram for the system, it is necessary to specify the onset and completion temperatures for the transitions of a series of mixtures, and it is evident that uncertainty in this selection arises from the generally rounded nature of the transition curves, due to premelting and other unidentified causes. In the present case the curves are sufficiently steep so that various extrapolation procedures, such as that indicated by dotted lines for the left-hand curve in Fig. 2, give results agreeing to within a few tenths of a degree.

The onset and completion temperatures were corrected for the contributions to the total transition widths which stem from the finite widths of the transition curves of the pure lipids. To the onset temperature, T_1, we have added the quantity $X_A\Delta T_{1A} + X_B\Delta T_{1B}$, where ΔT_{1A} is the temperature difference between the transition temperature of component A and the onset temperature of its transition, ΔT_{1B} is the corresponding quantity for component B, and X_A, X_B are the mole fractions of the components. Similarly, the observed completion temperature, T_2, is decreased by the amount $X_A\Delta T_{2A} + X_B\Delta T_{2B}$.

The phase diagram derived from observations on six mixtures of DMPC and DPPC is shown in Fig. 2B. Because of the corrections outlined in the preceding paragraph, the temperatures used to construct the phase diagram differ slightly from those which one would deduce directly from the observed transition curves. This phase diagram agrees reasonably well with that derived by Shimshick and McConnell (6) from observations with the electron spin label Tempo. The ideal diagram, shown

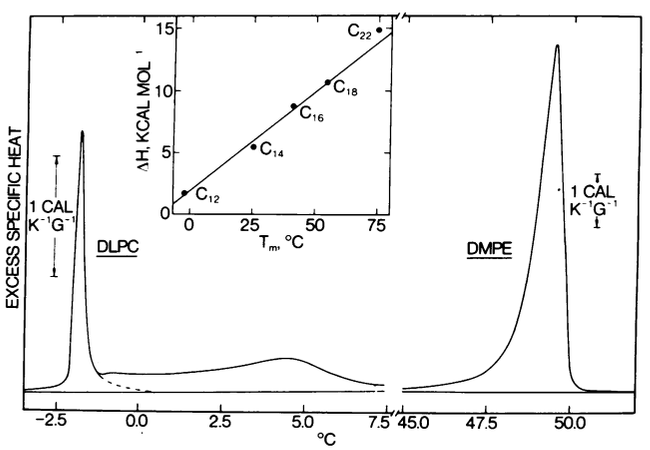

FIG. 1. Calorimetric transition curves for multilamellar suspensions of DLPC and DMPC. Insert: enthalpies for the upper transitions of phosphatidylcholines with acyl groups containing 12, 14, 16, 18, and 22 carbon atoms plotted against the transition temperature. The values for dibehenoylphosphatidylcholine are taken from Ladbrooke and Chapman (15).

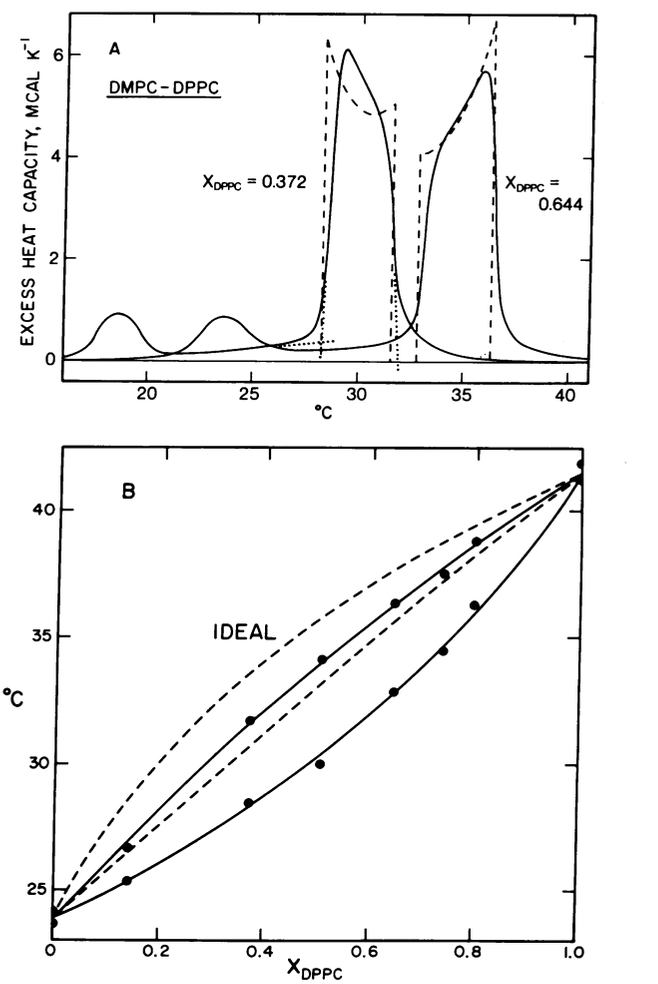

FIG. 2. (A) (—) Observed calorimetric transition curves for two mixtures of DMPC and DPPC; (- - -) transition curves calculated (see *text*) on the basis of the phase diagram in panel B. (B) (—) Phase diagram constructed from initiation and completion temperatures read from observed transition curves, corrected as described in the *text;* (- - -) ideal phase diagram calculated according to Eqs. 1 and 2. MCAL is a millicalorie.

as dashed curves in the figure, is computed by means of the relations (13)

$$X_B^{(l)} = (1 - \alpha)/(\beta - \alpha); \quad X_B^{(s)} = \beta X_B^{(l)} \qquad [1]$$

where the superscripts refer to the liquidus (upper) curve and the solidus (lower) curve, and the quantities α and β are defined as

$$\alpha = \exp\left[\frac{\Delta H_A}{R}\left(\frac{1}{T} - \frac{1}{T_A}\right)\right];$$

$$\beta = \exp\left[\frac{\Delta H_B}{R}\left(\frac{1}{T} - \frac{1}{T_B}\right)\right] \qquad [2]$$

where ΔH_A, ΔH_B are the heats of transition of the pure lipids and T_A, T_B are their absolute transition temperatures. It is evident that although this system shows complete miscibility in both liquid and solid phases, it is definitely not ideal. This is further indicated by the fact that the observed transition heats for the six mixtures are larger than the values calculated assuming zero heats of mixing by the factor 1.15 ± 0.06 (standard deviation).

It has been pointed out to us by Harden McConnell that the phase diagram gives all the information needed to calculate theoretical transition curves, if one neglects the heat effects associated with unmixing the "solid" components and mixing the "liquid" components during the transition†. It is evident that, for one mole of total lipid,

$$\frac{dH}{dT} = C_P \approx \Delta H_A \frac{d}{dT}(f^{(s)} X_A^{(s)}) + \Delta H_B \frac{d}{dT}(f^{(s)} X_B^{(s)}) \qquad [3]$$

where H and C_p are excess quantities, and the fraction, $f^{(s)}$, of the system present in the solid phase at a specified temperature during the transition is given by

$$f^{(s)} = \frac{X_B - X_B^{(l)}}{X_B^{(s)} - X_B^{(l)}} \qquad [4]$$

where $X_B^{(s)}$ and $X_B^{(l)}$ are the solid and liquid compositions that are in equilibrium at that temperature. Differentiation, as indicated in Eq. 3, leads to the result

$$C_P = \frac{X_A^{(l)} \Delta H_A + X_B^{(l)} \Delta H_B}{X_B^{(s)} - X_B^{(l)}} f^{(s)} \frac{dX_B^{(s)}}{dT} + \frac{X_A^{(s)} \Delta H_A + X_B^{(s)} \Delta H_B}{X_B^{(s)} - X_B^{(l)}} f^{(l)} \frac{dX_B^{(l)}}{dT} \qquad [5]$$

The quantities $X_B^{(l)}$ and $X_B^{(s)}$ are read from the phase diagram and the derivatives $dX_B^{(l)}/dT$ and $dX_B^{(s)}/dT$ are estimated graphically as the slopes of the liquidus and solidus curves.

The dashed curves in Fig. 2A are transition curves calculated by the above procedure from the phase diagram in Fig. 2B. It appears that the experimental curves are essentially rounded versions of the calculated curves. Although the calculation is to some extent circular in character in that the initiation and completion temperatures of the calculated curves necessarily coincide with the temperatures read from the phase diagram, we nevertheless consider that the reproduction of the asymmetry of the observed curves in the calculated curves is a strong indication of the validity of the phase diagram.

† The terms expressing the heats of mixing, if expressed in terms of mole fractions, will involve product terms of the form $X_A^m X_B^n$ for both the solid and liquid phases. As shown by Sturtevant and Lyons (14), these terms will probably be considerably simpler in form if volume fractions instead of mole fractions are used. Unfortunately there seems to be no direct way of determining excess enthalpies of mixing in bilayer systems since diffusion between separate bilayer domains is extremely slow.

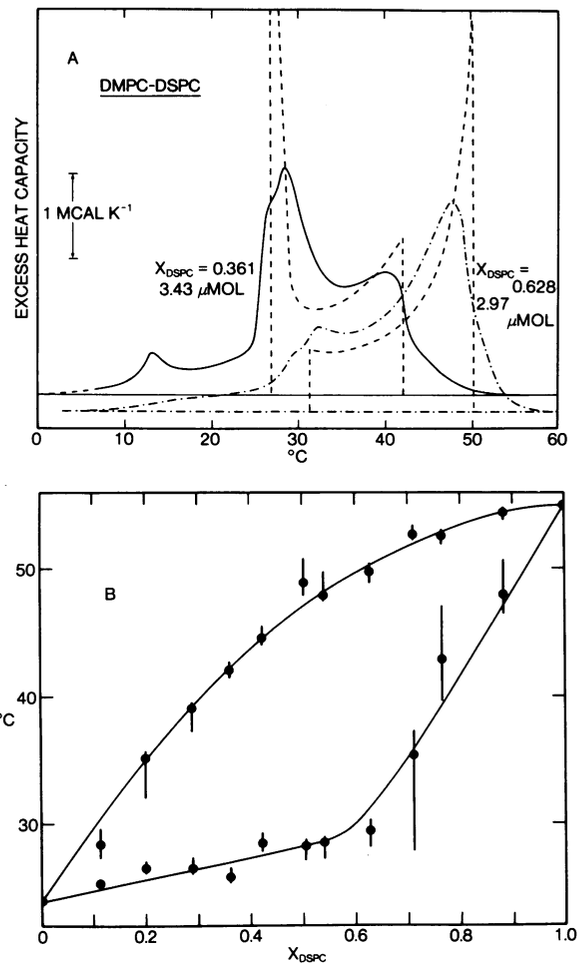

FIG. 3. (A) (—) and (- · -) Observed transition curves for two mixtures of DMPC and DSPC; (- - -) calculated transition curves (see *text*). (B) Phase diagram constructed from calorimetric transition curves.

It may be noted that the observed transition curves can, to well within experimental uncertainty, be resolved into two separate peaks. However this interpretation of the data, which would amount to postulating the existence of two solid phases, is contrary to the phase rule; with a total of three phases and two components, the system would have only one degree of freedom, and since this is taken up by the fixed pressure, isothermal melting would be required. This would mean at the least that either the solidus curve on the component A side of the diagram or the liquidus curve on the component B side, or both, would have to have horizontal portions, which is contrary to the observations.

DMPC–DSPC. This system, with hydrocarbon chains differing by four methylene groups, is considerably further removed from ideal than the system DMPC–DPPC. Transitions for two mixtures are shown in Fig. 3A. It is obvious that the selection of onset and completion temperatures for this system is considerably less precise than for DMPC–DPPC, especially since it appears probable that the lower transition of DSPC makes some contribution to what is taken as the main transition

of the mixture. The phase diagram‡ obtained for this system is shown in Fig. 3B, with estimates of the uncertainties of the selected temperatures being included. Again there is quite good agreement with the diagram for this system derived by Shimshick and McConnell (6) from the spin label experiments. These authors concluded that this system shows lateral separation into two phases in the solid state, but our calorimetric data do not support this conclusion since there is no region of isothermal melting.

The nonideality of this system, which is evident in the phase diagram, is indicated also in the observed enthalpies. Since it is impossible to resolve the lower transitions in this system, we have considered only the total enthalpies. These averaged 1.22 ± 0.04 (standard deviation) times the enthalpies expected on the basis of the amounts of the lipids present.

It should be emphasized that abnormal portions of transition curves, such as that between 5° and 25° for the mixture with $X_{DSPC} = 0.628$, are not, in the Privalov calorimeter, mere baseline aberrations, but reflect real heat absorptions taking place. Although in most cases we are unable to identify the causes of the enthalpy changes taking place, we do not believe that these unexplained effects detract from the general conclusion that for the most part these systems follow the requirements of the phase rule.

DMPE–DSPC. Since DMPE and DSPC have transition temperatures differing by only 5.5° (Table 1), the mixture transition curves in this system are only slightly broader than the individual lipid curves, and the corrections to the initiation and completion temperatures have a large effect. This makes the construction of a reliable phase diagram for this system rather difficult. Observations on nine mixtures, the transition curves for two of which are shown in Fig. 4A, were used to obtain the phase diagram given in Fig. 4B. As in the preceding cases, the mixture curves move steadily from lower to higher temperatures as the mole fraction of DSPC is increased, so that we again conclude that the system, although certainly not ideal, does not show appreciable solid state lateral phase separation in the temperature range studied. It is admittedly difficult to rule out the possibility of immiscibility at a mole fraction of either component less than 0.1 since the total temperature spread is so small.

Calculated transition curves for this system are shown in Fig. 4A. The calculated curves are much narrower than the observed curves because of the transition breadth corrections that were applied in constructing the phase diagram.

DLPC–DSPC. The calorimetric data for this system show that the chain length difference of 6 carbon atoms leads to a system so far from ideal that monotectic behavior is observed. This same conclusion was reached by Op den Kamp *et al.* (4). As seen in Fig. 5A, the peak for DLPC remains at constant temperature over nearly the entire concentration range, while that for DSPC moves continuously down in temperature. This indicates that DSPC is only very slightly soluble in DLPC in the gel phase while DLPC has significant solubility in DSPC. Evidently, as would be expected, the presence of impurity chains in a lipid bilayer that are shorter than the bulk of the chains is less perturbing to the structure than the presence of longer chains. From the DLPC peak areas it can be estimated that the solubility of DLPC in DSPC is 15 ± 5 mole percent at about 0°.

The phase diagram deduced for this system from the calorimetric results is shown in Fig. 5B. It was not determined just how far to the right side of the diagram the horizontal portion of the solidus curve extends. The dotted curve in Fig. 5A is a calculated transition curve for $X_{DSPC} = 0.498$ based on this phase diagram.

‡ The corrections to onset and completion temperature outlined above have been applied in this diagram also to the pure components, so that their transitions are represented as being isothermal.

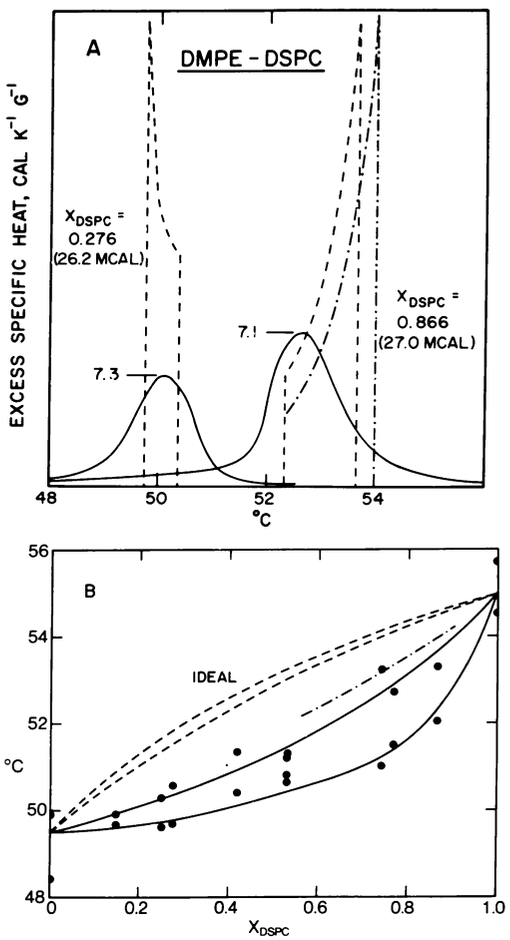

FIG. 4. (A) (—) Observed transition curves for two mixtures of DMPE and DSPC; (- - -) calculated transition curves (see *text*); (- · -) calculated transition curve based on the (- · -) curve in phase diagram (panel B). (B) (—) Phase diagram constructed from calorimetric transition curves; (- - -) ideal phase diagram calculated according to Eqs. [1] and [2].

CONCLUSIONS

As is to be expected from classical studies of small molecules, high sensitivity differential scanning calorimetry is a powerful method for studying the thermal properties of lipids in bilayer systems. This method is unique in that it has high precision and, at the same time, does not in any way perturb the system under study. In particular it provides a reliable procedure for detecting phase separation in the gel state of binary mixtures. The results presented here give strong support to a literal interpretation of phase diagrams for these systems, or, otherwise stated, to the applicability of the phase rule in cases where adequate experimental data are available.

Much additional work will be required to map out completely the minimum structural differences that can be expected to lead to lateral phase separation in either the gel or liquid crystalline states of multilamellar binary lipid mixtures. At present it appears that with saturated components a minimum difference in hydrocarbon chain length of six carbon

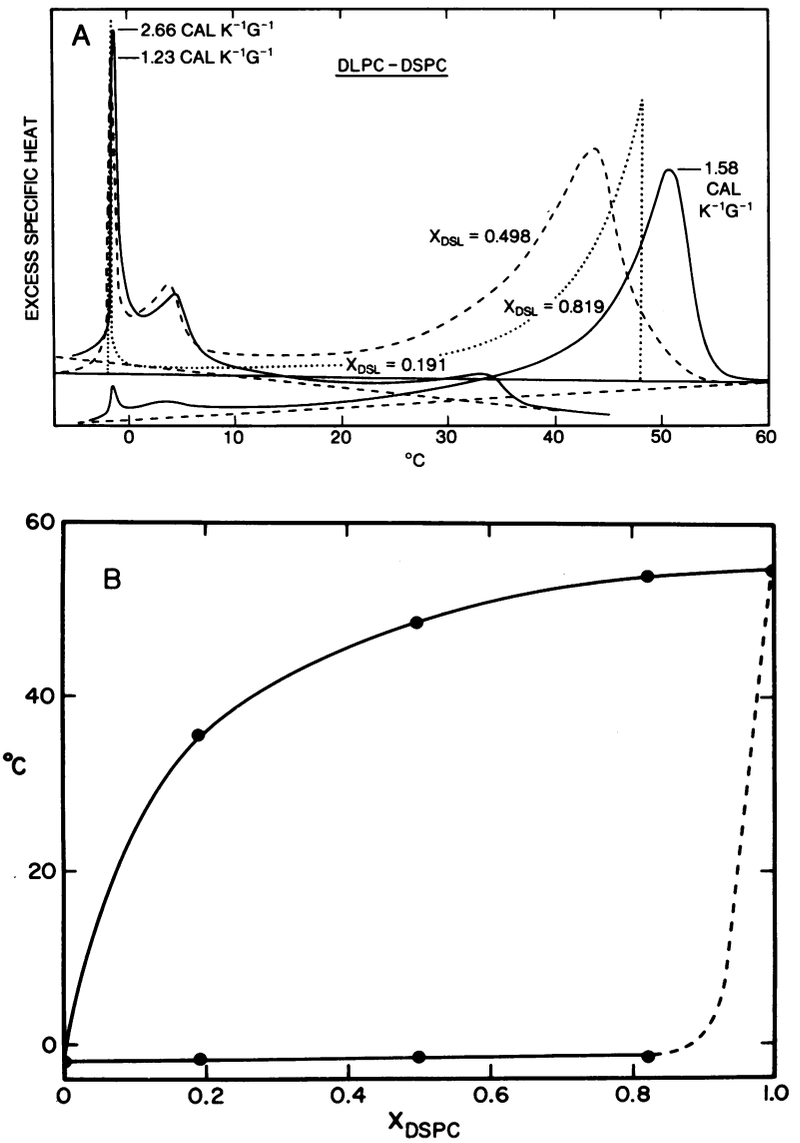

FIG. 5. (A) (Solid curves, dashed baselines) transition curves for DLPC–DSPC mixtures with X_{DSPC} = 0.191 and 0.819; (dashed curve, solid baseline) transition curve with X_{DSPC} = 0.498; (dotted curve) calculated transition curve for X_{DSPC} = 0.498. (B) Phase diagram constructed from calorimetric transition curves.

atoms is required, and that with a given chain length difference the substitution of phosphatidylethanolamine for phosphatidylcholine head groups in the lower melting component does not increase the tendency toward phase separation.

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