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DIFFERENTIAL MISCIBILITY PROPERTIES OF VARIOUS PHOSPHATIDYLCHOLINE/LYSOPHOSPHATIDYLCHOLINE MIXTURES

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Summary

Using enthalphy data from differential scanning calorimetry experiments and ^{13}C-NMR linewidths of specifically (N-Me-^{13}C)-labelled lipids, the miscibility properties of phosphatidylcholines and lysophosphatidylcholines in liposomal dispersions have been investigated. It was found that 16 : 0 lysophosphatidylcholine mixes homogeneously in 16 : 0/16 : 0 phosphatidylcholine bilayers. Mixtures of 16 : 0 lysophosphatidylcholine with 18 : 1_c/18 : 1_c phosphatidylcholine, of 18 : 1_c lysophosphatidylcholine with 16 : 0/16 : 0 phosphatidylcholine and of 18 : 1_c lysophosphatidylcholine with 18 : 1_c/18 : 1_c phosphatidylcholine exhibited immiscibility in the phosphatidylcholine gel state.

Introduction

Lysophospholipids, especially lysophosphatidylcholines, are known to occur as minor constituents in various cell membranes [1]. In phospholipid metabolism lysophospholipids are key intermediates [2]. Under various conditions lysophosphatidylcholines may induce morphological changes in cells [3], facilitate cell fusion [4], cause hemolysis [5—7] and affect the permeability properties of phosphatidylcholine liposomes [5,6,8,9]. The wedge shape of the lysophospholipid molecules suggests a possible common mechanism in these observations. In order to elucidate this mechanism several studies have been performed concerning the properties of mixed lysophospholipid/phospholipid systems. X-ray and light-scattering studies [8] on egg phosphatidylcholine-egg lysophosphatidylcholine dispersions have indicated that up to 40—50 mol% of the lyso compound all the lipid is arranged in a lamellar phase. However, the way phospholipids and lysophospholipids mix in this phase may show distinct

variations. Using differential scanning calorimetry, Blume et al. [10] found indications that an ether-desoxy lysophosphatidylcholine analog might induce or abolish phase separations, depending on the lipid or lipid mixture used. More precisely, Klopfenstein et al. [11] suggested that 16 : 0 lysophosphatidylcholine mixes homogeneously with 16 : 0/16 : 0 phosphatidylcholine. In contrast, a permeability study using ^{1}H-NMR led Lee and Chan [12] to suggest that in 14 : 0 lysophosphatidylcholine-14 : 0/14 : 0 phosphatidylcholine and 16 : 0 lysophosphatidylcholine-16 : 0/16 : 0 phosphatidylcholine vesicular bilayers a lateral phase separation exists in the gel state. In the liquid-crystalline state they suggest that the formation of ion channels consisting of clusters of four lysophosphatidylcholine molecules is likely. In order to check these opposing viewpoints, we have used differential scanning calorimetry and ^{13}C-NMR to compare the mixing properties of different lysophosphatidylcholines and phosphatidylcholines.

Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (16 : 0/16 : 0 phosphatidylcholine), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18 : 1_c/18 : 1_c phosphatidylcholine), 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (22 : 1_c/22 : 1_c phosphatidylcholine) were synthesized as described previously [13]. (*N-Me*-^{13}C)-labelled 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and (*N-Me*-^{13}C)-labelled 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine were synthesized according to de Kruijff et al. [14]. 1-Oleoyl-*sn*-3-phosphocholine (18 : 1_c lysophosphatidylcholine), 1-palmitoyl-*sn*-3-phosphocholine (16 : 0 lysophosphatidylcholine), *N-Me*-^{13}C)-labelled 1-oleoyl-*sn*-3-phosphocholine and (*N-Me*-^{13}C)-labelled 1-palmitoyl-*sn*-3-phosphocholine were obtained by enzymic hydrolysis using phospholipase A_2 from pig pancreas (a gift from Dr. B. Verhey). The lipids were greater than 99% pure as judged by thin-layer chromatography and occasionally gas chromatography. All other reagents used were analytical grades.

Methods

Lipids were mixed as chloroform solutions and subsequently the chloroform was evaporated under nitrogen. The samples were stored under vacuum overnight. For differential scanning calorimetry experiments, the lipids were dispersed in a 100 mM NaCl, 10 mM Tris-HCl, 0.2 mM EDTA buffer of pH 7.0 to a final concentration of about 100 mM by vortexing above the phase-transition temperatures of the lipids. About 15 μl of these dispersions, containing 1—2 μmol of lipid, was sealed in aluminium sample pans. Triplicate heating and cooling scans were run on a Perkin-Elmer DSC-2 apparatus with a scanning rate of 5 K/min at a sensitivity range of 1 mcal/s. Temperature and enthalpy calibration were performed with naphthalene, benzoic acid, biphenyl and water. The amount of phospholipid present in the sample pan was determined after perchloric acid destruction according to Fiske and SubbaRow [15].

Initially, lipids exhibiting phase transitions near or below 0°C were dispersed in a buffer containing 50% (v/v) ethyleneglycol to prevent the freezing of

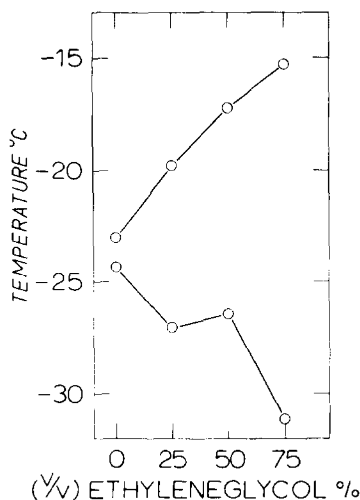

Fig. 1. Transition temperatures of 18 : 1_c/18 : 1_c phosphatidylcholine in buffers containing increasing amounts of ethyleneglycol. The upper trace represents temperatures from heating scans, the lower one temperatures from cooling scans.

water. However, experiments revealed that the presence of 50% (v/v) ethyleneglycol influences the thermotropic behavior of phospholipids to a variable extent, depending on the lipid species. Main features in this respect are: the abolishment of the pretransition of saturated phosphatidylcholines (i.e. 16 : 0/16 : 0 phosphatidylcholine); the introduction of an exothermic transition before the main transition upon heating dispersions of unsaturated phosphatidylcholines (i.e. 18 : 1_c/18 : 1_c phosphatidylcholine and 22 : 1_c/22 : 1_c phosphatidylcholine); an 50% increase in the transition enthalpy of the unsaturated phosphatidylcholines mentioned and to a lesser extent (some 25%) of 16 : 1/16 : 0 phosphatidylcholine and 16 : 0 lysophosphatidylcholine; a change in transition temperatures in heating and cooling scans, leading to hysteresis which is most profound in 18 : 1_c/18 : 1_c phosphatidylcholine and clearly illustrated in Fig. 1. Calorimetric behavior of 16 : 0 lysophosphatidylcholine and 18 : 1_c/18 : 1_c phosphatidylcholine mixtures was qualitatively not seriously affected by ethyleneglycol, but absolute enthalpies were again increased and transition temperatures were changed. Therefore in all further experiments described in this paper ethyleneglycol was omitted. When necessary, measurements were done with frozen or supercooled samples or the ice-water transition was allowed to take place isothermally.

The compositional values of the lipid mixtures are accurate to ±1.0–1.5 mol%. The transition temperatures, which are taken as the intercept of the predominant slope of the first appearing arm of the transition peaks with the baseline, have an absolute S.D. ranging from 0.1°C for the sharp peaks to 0.5°C for the less well-defined peaks. Enthalpy values have an absolute S.D. varying from 0.5 kcal/mol for the highest values to 0.1 kcal/mol for the lowest values.

^{13}C-NMR measurements were performed on a Bruker 360 WS spectrometer operating at a frequency of 90.5 MHz using 1.3 W continuous proton noise

decoupling, a 90° pulse angle with a 1.0 s interpulse time and a spectral width of 20 kHz with 16K data points. Accumulated free induction decays were obtained from 500 to 2000 transients on 1.5-ml samples containing 20–70 μmol lipid. Dispersions were prepared as described above, but the buffer now contained 20% (v/v) ^2H_2O. The measured *N-Me*-^{13}C linewidths were estimated to have a S.D. of about 5 Hz. The error in the linewidths of the broad peaks, which were obtained after deconvolution of the composite peaks, is about 10 Hz.

Results and Discussion

Differential scanning calorimetry of mixtures of 16 : 0 lysophosphatidylcholine with 16 : 0/16 : 0 phosphatidylcholine or 18 : 1_c/18 : 1_c phosphatidylcholine

In the earlier study of Klopfenstein et al. [11] on the calorimetric behavior of mixtures of 16 : 0 lysophosphatidylcholine and 16 : 0/16 : 0 phosphatidylcholine ethyleneglycol was used in the buffer as an antifreeze. This may have serious effects on the thermotropic behavior of lipids (see Materials and Methods). Therefore we have repeated these measurements in an aqueous buffer. Because micellar 16 : 0 lysophosphatidylcholine undergoes a transition at about 3°C [11], close to the ice-water transition, and the transitions in micellar solutions were found to have a strong hysteresis, two experimental procedures were employed to record the heating scans of the samples. In the first procedure the sample was cooled in the calorimeter to approximately −8°C, thus supercooling the aqueous buffer, and then the heating scan was recorded. In the second procedure the sample was first frozen in the calorimeter at −33°C, then heated to 2°C to melt isothermally the ice. It was subsequently supercooled to −8°C and then the heating scan was recorded. The heating scans of the lipid mixtures obtained under these two conditions are shown in Fig. 2A.

The transition enthalpies and temperatures are summarized in Fig. 3A. Enthalpies derived from transitions in cooling scans were found to be similar to those from heating scans recorded with the first procedure. The main transition of pure 16 : 0/16 : 0 phosphatidylcholine occurred at 40.2 ± 0.2°C with a ΔH of 8.7 ± 0.3 kcal/mol and is preceded by a pretransition occurring at 29.5 ± 0.3°C with a ΔH of 1.7 kcal/mol, in agreement with other studies [16,17]. The hysteresis in a micellar solution of 16 : 0 lysophosphatidylcholine is such that no transition could be detected employing the first procedure, nor was one present in the cooling scans.

Only when the second procedure was used a transition was found at 3.0 ± 0.2°C with a ΔH of 4.0 ± 0.1 kcal/mol, which is closely similar to the value of 4.5 kcal/mol found in ethyleneglycol-containing buffers [11]. The hysteresis is most likely caused by the difficult crystallization of the acyl chains in a micelle. The shape and/or size of the micelle will probably have to change during the transition which apparently is facilitated by the ice-water transition.

For 16 : 0 lysophosphatidylcholine concentration up to 40 mol% only the sharp main transition of 16 : 0/16 : 0 phosphatidylcholine is observed (Fig. 2A). In this concentration range the two lipids are present in mixed bilayers

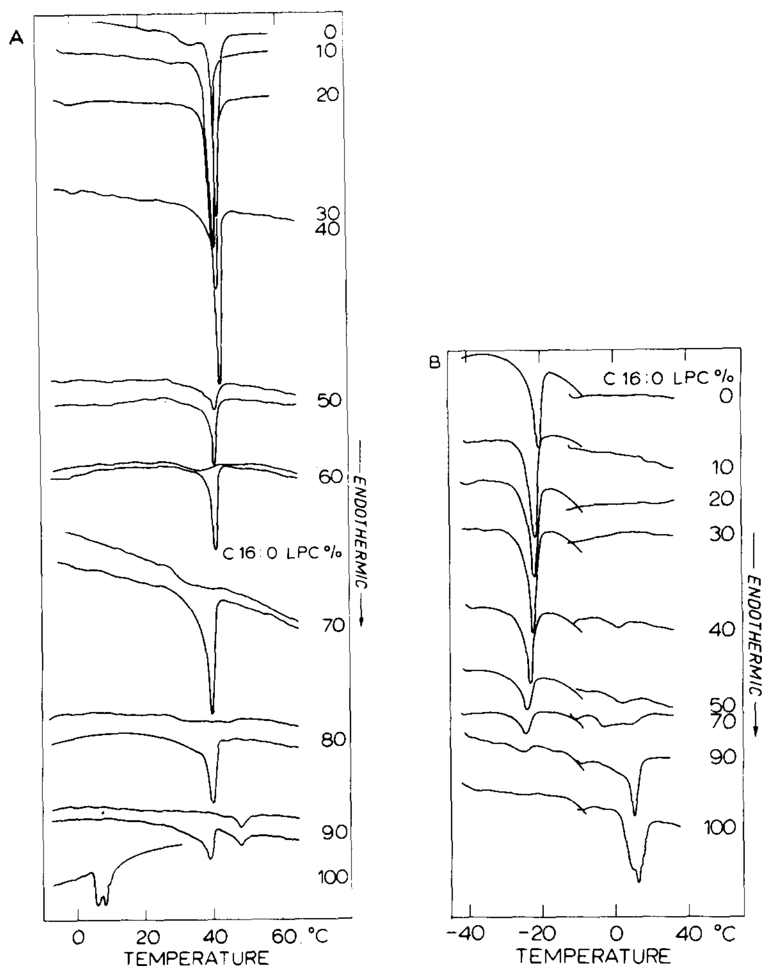

Fig. 2. (A) Calorimetric heating scans of mixtures of 16 : 0 lysophosphatidylcholine (C16 : 0 LPC) and 16 : 0/16 : 0 phosphatidylcholine. For 16 : 0 lysophosphatidylcholine concentrations from 50 to 90 mol% two scans are shown. The upper scan is for the first procedure mentioned in the text, and the lower for the second procedure. (B) Calorimetric heating scans of mixtures of 16 : 0 lysophosphatidylcholine (C16 : 0 LPC) and 18 : 1_c/18 : 1_c phosphatidylcholine.

(van Echteld, C.J.A., de Kruijff, B., Mandersloot, J.G. and de Gier, J., unpublished results). Information concerning the mixing properties of both components in the bilayer can be obtained from the ΔH values of the phase transition (Fig. 3A). if only the 16 : 0/16 : 0 phosphatidylcholine in the mixtures undergoes a phase transition with a ΔH value similar to the one of pure 16 : 0/16 : 0 phosphatidylcholine, then enthalpies which are proportional to the fraction of 16 : 0/16 : 0 phosphatidylcholine in the sample should be observed.

In this case the experimental points should fit a straight line connecting the ΔH value of 100% 16 : 0/16 : 0 phosphatidylcholine with $\Delta H = 0$ for 0% 16 : 0/16 : 0 phosphatidylcholine. It is clear from Fig. 3A that the observed ΔH values are significantly higher. Mixtures containing 10 or more mol% 16 : 0 lysophosphatidylcholine lack the pretransition. The relatively high ΔH value of

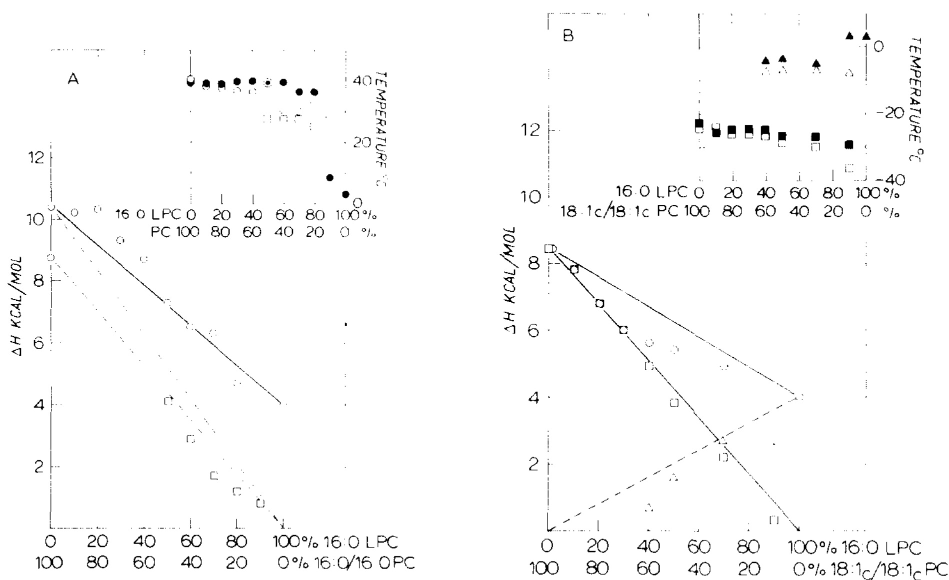

Fig. 3. (A) Transition enthalpies and temperatures of mixtures of 16 : 0 lysophosphatidylcholine and 16 : 0/16 : 0 phosphatidylcholine. ○, total enthalpy values obtained via the second procedure mentioned in the text. □, enthalpy values obtained via the first procedure in those mixtures where the two procedures yielded different values. · · · · · ·, values proportional to the molar fractions of the enthalpy change at the main transition of the pure phosphatidylcholine. Insert: ●, transition temperature from heating scan using the second procedure. ○, transition temperature from cooling scan. □, transition temperature from heating scan using the first procedure. (B) Transition enthalpies and temperatures of 16 : 0 lysophosphatidylcholine and 18 : 1_c/18 : 1_c phosphatidylcholine. □, enthalpy values from peaks in the low-temperature region. △, enthalpy values from peaks in the high-temperature region. The anomalous value found at 90 mol% 16 : 0 lysophosphatidylcholine arises from the large hysteresis which leads to severe difficulties in determining the lipid phase transition because of the ice-water transition. ○, sum of both enthalpies. Insert: ■, transition temperature from heating scan in the low-temperature region. □, transition temperature from cooling scan in the low-temperature region. ▲, transition temperature from heating scan in the high-temperature region. △, transition temperature from cooling scan in the high-temperature region.

the 10 mol% 16 : 0 lysophosphatidylcholine-containing mixture makes it tempting to suggest that the energy required for the pretransition is now part of the main transition. Therefore the argument can be raised whether the enthalpy of the pretransition should be taken into account in a calculation of expected ΔH values of the mixtures. However, it is clear from Fig. 3A that the experimental ΔH values are still significantly higher than these predicted ones, assuming only 16 : 0/16 : 0 phosphatidylcholine is contributing to the transition enthalpy. The alternative possibility is that 16 : 0 lysophosphatidylcholine also melts in the bilayer at the same temperature as 16 : 0/16 : 0 phosphatidylcholine. Assuming that the transition enthalpy of 16 : 0 lysophosphatidylcholine in the bilayer is identical to the value observed in the micelle, this would then predict ΔH values fitting a line connecting the ΔH values of the pure components.

The experimental points are close to these predicted values. The data therefore suggest that in the 0—40 mol% 16 : 0 lysophosphatidylcholine concentration range the acyl chain of the lysocompound is melting simultaneously with

the acyl chains of 16 : 0/16 : 0 lysophosphatidylcholine and the two components are homogeneously distributed in the bilayer, which is in agreement with Klopfenstein's results. Further support for this suggestion is found in the transition temperatures. Lee [18] has calculated phase diagrams for binary lipid mixtures with complete miscibility in liquid and solid phases to consist of convex solidus and liquidus curves, when the lower melting component has a fairly low transition enthalpy and the higher melting one a fairly high transition enthalpy. When restricting to the situation below 40 mol% 16 : 0 lysophosphatidylcholine the convex curves are reflected in the hardly changing transition temperatures in this concentration range (see insert Fig. 3A).

At 16 : 0 lysophosphatidylcholine concentrations higher than 40 mol% the samples become visibly clearer, because the lyso compound disrupts the bilayer leading to the formation of small aggregates (van Echteld, C.J.A., de Kruijff, B., Mandersloot, J.G. and de Gier, J., unpublished results). In the calorimetric scans this is mainly manifest in an increasingly larger hysteresis in the phase transition (Fig. 3A). The lower enthalpy values, obtained using the first scanning procedure, reflect lipid that still crystallizes easily, and is thus most likely arranged in a bilayer; while the higher enthalpy values, obtained from the second procedure, are the sums of these low values with a part originating from lipid forced to crystallize in a micelle or in a bilayer configuration. For 16 : 0 lysophosphatidylcholine concentrations up to 90 mol% there are single transitions detected in the 16 : 0/16 : 0 phosphatidylcholine region, with enthalpies corresponding to the sum of both components. This strongly suggests that also in this mixed-micelle region the palmitic acid chain of the lyso compound melts together with those of the diacyl compound, indicating a homogeneous mixing of both components.

To avoid loss of information due to the ice-water transition, dispersions of mixtures of 16 : 0 lysophosphatidylcholine and 18 : 1_c/18 : 1_c phosphatidylcholine were scanned until the ice started to melt, next heated to 2°C to let the ice melt, subsequently supercooled and then scanned further. The heating scans of these mixtures are shown in Fig. 2B. Enthalpies derived from both heating and cooling scans, which yielded similar results, are summarized in Fig. 3B, together with the transition temperatures. The transition of the pure 18 : 1_c/18 : 1_c phosphatidylcholine occurred at −23.0 ± 0.2°C with a ΔH of 8.4 ± 0.3 kcal/mol in fair agreement with previous results [19]. For 16 : 0 lysophosphatidylcholine concentrations up to 30 mol% only the transition of 18 : 1_c/18 : 1_c phosphatidylcholine is observed. In contrast to the previous system, the experimental ΔH values in Fig. 3B lie very close to the line connecting the ΔH value of 100% 18 : C_c/18 : 1_c phosphatidylcholine with $\Delta H = 0$ for 0% 18 : 1_c/18 : 1_c phosphatidylcholine. This therefore suggests that only 18 : 1_c/18 : 1_c phosphatidylcholine undergoes a phase transition in the mixtures, with an enthalpy similar to that of the pure compound. This would thus imply immiscibility with the lysocompound, at least in the gel state. However, a transition in the region of the 16 : 0 lysophosphatidylcholine transition, which would be expected for the case of immiscibility, is not present. An explanation for this phenomenon might be that 16 : 0 phosphatidylcholine is present in the 18 : 1_c/18 : 1_c phosphatidylcholine bilayer in small aggregates which have such a low transition cooperativity that the signal is too broad to be detected and in

such a way that the transition cooperativity of the 18 : 1_c/18 : 1_c phosphatidylcholine is hardly affected. Above 30 mol% 16 : 0 lysophosphatidylcholine there is a second transition manifest in the high-melting part of the scans indicating that a phase separation occurs in the region where mixed micelles are formed (van Echteld, C.J.A., de Kruijff, B., Mandersloot, J.G. and de Gier, J., unpublished results). The enthalpy missing in the 16 : 0 lysophosphatidylcholine transition at 40 mol% 16 : 0 lysophosphatidylcholine might reflect 16 : 0 lysophosphatidylcholine still present in small aggregates in the bilayer, which therefore is not observable.

Differential scanning calorimetry of mixtures of 18 : 1_c lysophosphatidylcholine with 16 : 0/16 : 0 phosphatidylcholine or 18 : 1_c/18 : 1_c phosphatidylcholine

The reported different lytic activity [5] of the less common 18 : 1_c lysophosphatidylcholine may result from a different mixing behavior compared with the 16 : 0 lysophosphatidylcholine. Therefore we have performed a similar calorimetric study on mixtures which include 18 : 1_c lysophosphatidylcholine. The calorimetric analysis of dispersions of lipid mixtures which include 18 : 1_c lysophosphatidylcholine is hampered by the fact that for 18 : 1_c lysophosphatidylcholine micelles the phase transition, which one might expect to occur some 40 degrees below that of 18 : 1_c/18 : 1_c phosphatidylcholine (comparing the palmitoyl compounds), could not be detected due to instrumental limitations.

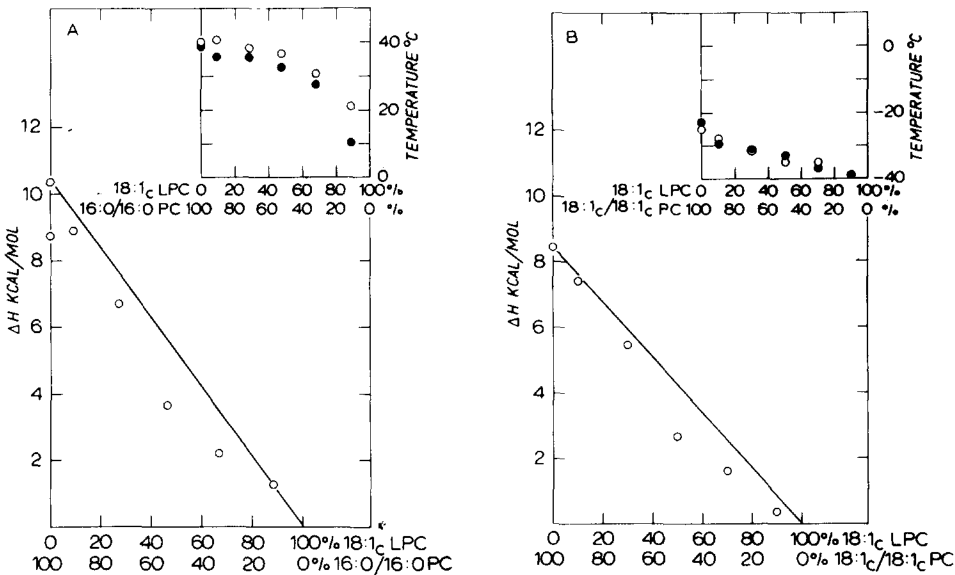

Fig. 4. (A) Transition enthalpies and temperatures of mixtures of 18 : 1_c lysophosphatidylcholine and 16 : 0/16 : 0 phosphatidylcholine. ○, enthalpy values of the observed peak. Insert: ●, transition temperature from heating scan. ○, transition temperature from cooling scan. (B) Transition enthalpies and temperatures of mixtures of 18 : 1_c lysophosphatidylcholine and 18 : 1_c/18 : 1_c phosphatidylcholine. ○, enthalpy values of the observed peak. Insert: ●, transition temperature from heating scan. ○, transition temperature from cooling scan.

Transition enthalpies derived from both heating and cooling scans of dispersions of mixtures of 18 : 1_c lysophosphatidylcholine and 16 : 0/16 : 0 phosphatidylcholine were similar and are summarized in Fig. 4A. The insert shows the transition temperatures. Here too, the pretransition was abolished in the mixtures with the lyso compound. Unlike the system with 16 : 0 lysophosphatidylcholine a profound broadening of the transition peak, indicating a loss of cooperativity, was observed at the lowest concentration of 10 mol% 18 : 1_c lysophosphatidylcholine. Using the same argument as before concerning the pretransition enthalpy, one sees from Fig. 4A that the lyso compound is not contributing to the transition enthalpy. Hence these results suggest immiscibility of the two lipid species, at least in the gel state.

The calorimetric properties of dispersions of mixtures of 18 : 1_c lysophosphatidylcholine and 18 : 1_c/18 : 1_c phosphatidylcholine are essentially the same as those above for mixtures of 18 : 1_c lysophosphatidylcholine and 16 : 0/16 : 0 phosphatidylcholine. There is also a similar broadening of the transition peaks at low concentrations of the lyso compounds. The transition enthalpies, which are shown in Fig. 4B, show the same features. Hence these results also suggest immiscibility in the gel state. The way in which 18 : 1_c lysophosphatidylcholine induces non-bilayer phases shows distinct differences among these systems and is different from the way 16 : 0 lysophosphatidylcholine acts (van Echteld, C.J.A., de Kruijff, B., Mandersloot, J.G. and de Gier, J., unpublished results). Hence, due to these structural complications, conclusions about the miscibility properties of the 18 : 1_c lysophosphatidylcholine-containing mixtures cannot be drawn exclusively for the liposomal bilayer from the calorimetric data.

^{13}C-NMR

The ^{13}C NMR linewidth of the choline *N*-methyl carbons in phosphatidylcholines changes abruptly upon passing the gel to liquid-crystalline phase transition [20]. Hence specific labelling enables one to determine the individual phase behavior of lipids in lipid mixtures.

Dispersions of mixtures of 70 mol% 16 : 0/16 : 0 phosphatidylcholine and 30 mol% lysophosphatidylcholine, in which all the lipid is present in a bilayer configuration, were prepared with the choline *N*-methyl groups of the two lipid species alternately ^{13}C labelled. Hence one experiment yielded the (*N-Me*-^{13}C) linewidths of 16 : 0/16 : 0 phosphatidylcholine, while the other one yielded the (*N-Me*-^{13}C) linewidths of 16 : 0 lysophosphatidylcholine in a mixture of the same composition. Both experiments covered a temperature range of 0—60°C. The data obtained are summarized in Fig. 5A. As is clearly seen from this figure, the sharp decrease in linewidth of the signals originating from either component occurs in the same temperature region which is close to the phase-transition temperature of 16 : 0/16 : 0 phosphatidylcholine. These results strongly suggest a simultaneous melting of both components in this bilayer, in full agreement with the calorimetric data.

The other mixture in which a phase transition occurs above 0°C and hence allows the same kind of study is the 16 : 0/16 : 0 phosphatidylcholine/18 : 1_c lysophosphatidylcholine mixture. However, in experiments with mixtures containing 30 mol% 18 : 1_c lysophosphatidylcholine essentially narrow peaks were found for both lipid components. These narrow peaks are indicative of

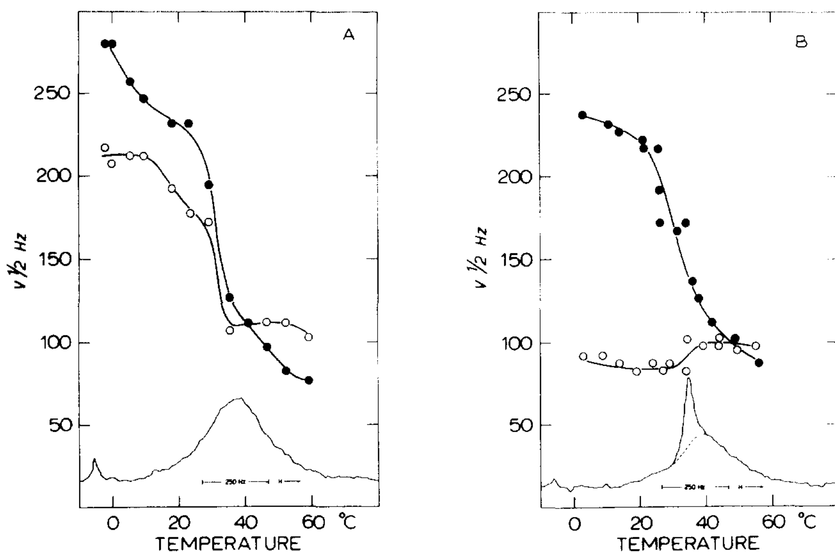

Fig. 5. (A) *N-Me*-^{13}C linewidths of 16 : 0/16 : 0 phosphatidylcholine (●) and 16 : 0 lysophosphatidylcholine (○) in a mixture containing 30 mol% 16 : 0 lysophosphatidylcholine. The insert shows as a typical example of *N-Me*-^{13}C resonance of 16 : 0/16 : 0 phosphatidylcholine at 10°C. (B) *N-Me*-^{13}C linewidths of the broad line of the resonances of 16 : 0/16 : 0 phosphatidylcholine (●) and 18 : 1_c lysophosphatidylcholine (○) in a mixture containing 10 mol% 18 : 1_c lysophosphatidylcholine. The insert shows as a typical example the composite *N-Me*-^{13}C resonance of 16 : 0/16 : 0 phosphatidylcholine at 10°C.

small lipid structures. Because we were interested in the mixing behavior of these two lipid species in a liposomal bilayer we reduced the 18 : 1_c lysophosphatidylcholine concentration to 10 mol%. In this case signals were obtained which were clearly a superposition of a broad and a narrow line, as is shown in the insert of Fig. 5B.

The contribution of the narrow component to the total signal of 16 : 0/ 16 : 0 phosphatidylcholine increased upon passing the phase transition, while for 18 : 1_c lysophosphatidylcholine it decreased. To obtain information on the mixing behavior of both lipid species in the liposomal bilayer we deconvoluted the composite spectra into a broad and a narrow line with the aid of simulated spectra. The linewidths of the broad lines were then determined, which are summarized in Fig. 5B. It is clearly seen that the sharp simultaneous decrease in linewidth, as was seen in Fig. 5A, is absent in this system. This absence suggests a lateral phase separation of both bilayer components below the phase transition, even at this relatively low concentration of 18 : 1_c lysophosphatidylcholine, reinforcing the conclusion drawn from the calorimetric data.

Conclusions

Information on the mixing behavior of lipids in lipid dispersions is obtained in this study from enthalpy data and ^{13}C-NMR linewidths, because we are not able to construct an entire phase diagram for the mixtures that we have investigated due to structural transitions and hysteresis.

The situation with relatively low lysophosphatidylcholine concentrations is

emphasized in this study, reflecting the situation commonly encountered in biological membranes. When the four mixtures studied are compared in this low lysophosphatidylcholine concentration range, different situations are found. The conclusion that 16 : 0 lysophosphatidylcholine mixes homogeneously both below and above the phase-transition temperature in 16 : 0/16 : 0 phosphatidylcholine bilayers agrees with the conclusion of Klopfenstein et al. [11]. Therefore the suggestion of Lee and Chan [12] that lateral phase separation occurs in the gel state of 16 : 0/16 : 0 phosphatidylcholine seems unlikely. However, their conclusions are based on experiments with sonicated vesicles, which might exhibit different mixing properties due to their high curvature. In the other mixtures immiscibility is found in the gel state of the phosphatidylcholines. With the techniques used, or with any other known technique it is difficult to derive information on the mixing behavior of the lyso compounds in the liquid-crystalline matrix of these mixtures. Differences among these mixtures are found in the different ways, which will be further investigated, the lyso compounds induce small lipid structures. Regarding the different miscibility properties of the lipid mixtures as found in this study, it is tempting to speculate on the lysophospholipid distribution in biomembranes being a function of the fatty acid composition of a membrane. For instance, a different lysophospholipid distribution might help to explain the different lytic activity of different lyso compounds and the temperature dependence of hemolysis.

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