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A calorimetry and deuterium NMR study of mixed model membranes of 1-palmitoyl-2-oleylphosphatidylcholine and saturated phosphatidylcholines

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Binary phase diagrams have been constructed from differential scanning calorimetry (DSC) data for the systems 1-palmitoyl-2-oleylphosphatidylcholine (POPC)/dimyristoylphosphatidylcholine (DMPC), POPC/dipalmitoylphosphatidylcholine (DPPC) and POPC/distearoylphosphatidylcholine (DSPC). Mixtures of POPC with DMPC exhibit complete miscibility in the gel and liquid crystalline states. Mixtures of POPC with DPPC or with DSPC exhibit gel phase immiscibility over the composition range 0–75% DPPC (or DSPC). These results, when taken together with previous studies of mixtures of phosphatidylcholines, are consistent with the hypothesis that PCs whose order-disorder transition temperatures (T_m values) differ by less than 33 deg. C exhibit gel state miscibility. Those whose T_m values differ by more than 33 deg. C exhibit gel state immiscibility. ^{2}H-NMR spectroscopy has been used to further study mixed model membranes composed of POPC and DPPC, in which either lipid has been labeled with deuterium in the 2-, 10- or 16-position of the palmitoyl chain(s) or in the *N*-methyls of the choline head group. POPC/DPPC mixtures in the liquid crystalline state are intermediate in order between pure POPC and DPPC at the same temperature. The POPC palmitoyl chain is always more disordered than the palmitoyl chains of DPPC in liquid crystalline POPC/DPPC mixtures. This is attributed to the fact that a POPC palmitoyl chain is constrained by direct bonding to have at least one oleyl chain among its nearest neighbors, while a DPPC palmitoyl chain must have at least one neighboring palmitoyl chain. When liquid crystalline POPC, DPPC and POPC/DPPC mixtures are compared at a reduced temperature (relative to the acyl chain order-disorder transition), POPC/DPPC mixtures are more disordered than predicted from the behavior of the pure components, in agreement with enthalpy data derived from DSC studies. Within the temperature range of the broad phase transition of 1:1 POPC/DPPC, a superposition of gel and liquid crystalline spectra is observed for 1:1 POPC/[^{2}H]DPPC, while 1:1[^{2}H]POPC/DPPC exhibits only a liquid crystalline spectrum. Thus, at temperatures within the phase transition region, the liquid crystalline phase is POPC-rich and the gel phase is DPPC-rich. Comparison of the liquid crystalline quadrupole splittings within the thermal phase transition range suggests that mixing of the residual liquid crystalline POPC and DPPC is highly non-ideal.

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Abbreviations: DSC, differential scanning calorimetry; PC, phosphatidylcholine; DMPC, dimyristoylPC; DLPC, dilauroylPC; DOPC, dioleylPC; DPPC, dipalmitoylPC; DSPC, distearoylPC; POPC, 1-palmitoyl-2-oleylPC; [2-^{2}H$_2$]POPC, 1-[2-^{2}H$_2$]palmitoyl-2-oleylPC; [2,2'-^{2}H$_4$]DPPC, di([2-^{2}H$_2$]palmitoyl)PC; [10-^{2}H$_2$]POPC, 1-([10-^{2}H$_2$]palmitoyl)-2-oleylPC; [10,10'-^{2}H$_4$]DPPC, di([10-^{2}H$_2$]palmitoyl)PC; [16-^{2}H$_3$]POPC, 1-([16-^{2}H$_3$]palmitoyl)-2-oleylPC; [16,16'-^{2}H$_6$]DPPC, di([16-^{2}H$_3$]palmitoyl)PC; [*N-Me*-^{2}H$_9$]DPPC, dipalmitoylphosphatidyl[*N-Me*-^{2}H$_9$]choline; T_m, order-disorder transition temperature; T_o, transition onset temperature; T_f, transition completion temperature.

Introduction

Biological membranes from a variety of organisms have been shown to undergo lipid acyl chain order-disorder transitions. Model membranes composed of synthetic phospholipids have been extensively studied in an effort to obtain insight into such biomembrane phase transitions. Single component model membranes exhibit sharp acyl chain order-disorder transitions which can be characterized using a variety of physical approaches. Single component systems, of course, are poor models for complex membranes, and various binary mixtures of synthetic phospholipids have also been studied. Phase diagrams have been obtained for a variety of binary phospholipid mixtures and have demonstrated that certain combinations exhibit total miscibility in the gel phase, while others exhibit gel phase immiscibility (lateral phase separations) [1–6]. For example, DMPC/DPPC mixtures display almost ideal miscibility, while DLPC/DSPC or DOPC/DPPC mixtures clearly undergo gel state phase separations [4,7–9]. Quantitative analysis of the shape of such binary phase diagrams has indicated that even for those cases which show gel state miscibility, mixing is non-ideal in both gel and liquid crystalline phases [10].

None of the well-studied homogeneous saturated phosphatidylcholines is found in significant quantities in natural membranes. Natural phospholipids are mixed chain species which usually contain a saturated acyl chain in the glycerol *sn*-1 position and an unsaturated chain in the *sn*-2 position. Egg yolk phosphatidylcholine, for instance, is approx. 70% POPC [11]. In the present study, we have investigated the interactions of synthetic POPC with the saturated phosphatidylcholines DMPC, DPPC and DSPC. These studies were undertaken to determine the degree to which the behavior of a common mixed-acyl phospholipid (POPC) resembles that of the well-studied synthetic saturated phospholipids. We are particularly interested in the interactions of POPC with saturated phosphatidylcholines such as DPPC and DSPC because of their high order-disorder transition temperatures (T_m values), 42 and 55°C, respectively. While it is unlikely that phospholipids exhibiting such high T_m values occur in significant quantities in nature, high-melting glycolipids certainly do. Cerebrosides, for example, make up 20–25% of the lipid of central nervous system myelin [12] and have a T_m of approximately 67° [13]. It is thus of interest to determine the extent to which relative acyl chain composition alone affects the miscibility of lipids. A firm understanding of this behavior is a necessary prerequisite for the understanding of more complex phenomena such as head group-mediated clustering of glycolipids, which may be of real functional significance.

Materials and Methods

[2,2-$^2H_2$]Palmitate and [10,10-$^2H_2$]palmitate were synthesized by the method of Tulloch [14]. [16-$^2H_3$]Palmitate was purchased from Serdary Labs (London, Ontario) and was found to be pure by thin-layer chromatography and mass spectroscopy. DMPC and DPPC were synthesized by acylation of glycerylphosphorylcholine with the acyl imidazole of the appropriate deuterated or non-deuterated fatty acid [15]. DSPC was purchased from the Sigma Chemical Company, St. Louis, MO. [*N-Me*-$^2H_9$]Choline was synthesized by reaction of C^2H_3I with ethanolamine [16] and was purified by ion-exchange chromatography. The deuterated choline was coupled to dipalmitoylphosphatidic acid as previously described [17]. POPC was synthesized by phospholipase A_2 digestion of DPPC and reacylation, according to Gupta et al. [18]. All lipids were purified by silicic acid chromatography and exhibited only one spot on thin-layer chromatography in $CHCl_3$/CH_3OH/H_2O (65 : 25 : 4).

For DSC, lipid mixtures in 2 : 1 $CHCl_3$/CH_3OH solution were transferred to Perkin-Elmer DSC pans (50 μl capacity), dried under N_2 and desiccated overnight under vacuum. Distilled deionized water (30 μl) was added, and the pans were sealed. The lipid concentration was approx. 14 wt.%. Scanning calorimetry studies were carried out using a Perkin-Elmer DSC-2 scanning calorimeter. All reported DSC data were collected from heating runs at 2.5 deg. C/min, which followed cooling runs at 2.5 deg. C/min or 5 deg. C/min.

Lipid samples for ^{2}H-NMR were dried from organic solvent solution and desiccated under vacuum overnight. The samples were lyophilized

twice from deuterium-depleted H_2O (Sigma Chemical Company, St. Louis, MO) and finally suspended in deuterium-depleted H_2O at concentrations which ranged from 60 to 200 μmol/ml. ^{2}H-NMR was carried out on a Bruker HX-270 spectrometer operating at a deuterium frequency of 41.4 MHz with single 90° pulses of 25 μs duration. Spectra were obtained without sample spinning or proton decoupling.

Results

DSC

In Fig. 1 are presented scanning calorimetry runs of multilamellar dispersions of POPC, DMPC, DPPC and DSPC and of 1:1 mixtures of POPC with these three saturated phosphatidylcholines. POPC exhibits an acyl chain order-disorder transition with peak maximum (T_m) at −3°C, as shown in Fig. 1a. The enthalpy of this transition is 4.9 kcal/mol. Pure DMPC, DPPC and DSPC dispersions exhibit the well-described thermal pretransition and order-disorder transition at the appropriate characteristic temperatures (Fig. 1c, e, g). An equimolar POPC/DMPC dispersion exhibits a broad double-peaked transition at approx. 10°C, a temperature intermediate between the T_m values of the pure components (Fig. 1b). In Fig. 1d is presented the thermal behavior of an equimolar mixture of POPC and DPPC. This dispersion exhibits a very broad asymmetric transition which spans the range from −4 to 35.3°C. The thermal behavior of an equimolar POPC/DSPC dispersion is presented in Fig. 1f. In this case, two maxima are clearly observed, and the transition spans the broad range −4 to 50.8°C.

DSC traces were obtained for POPC/DMPC mixtures containing 0, 10, 25, 40, 50, 60, 75, 90

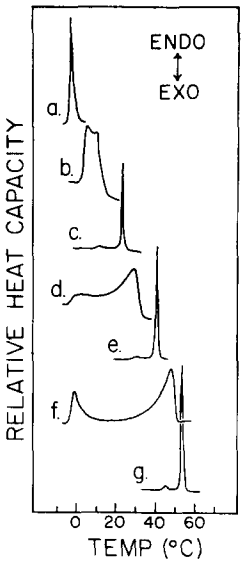

Fig. 1. DSC traces of multilamellar dispersions of (a) POPC, (b) 1:1 POPC/DMPC, (c) DMPC, (d) 1:1 POPC/DPPC, (e) DPPC, (f) 1:1 POPC/DSPC and (g) DSPC.

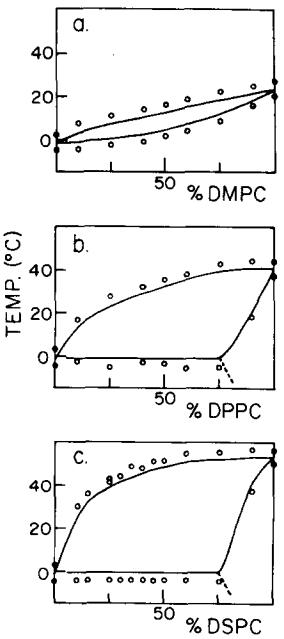

Fig. 2. Transition onset and completion temperatures as a function of lipid composition for (a) POPC/DMPC, (b) POPC/DPPC and (c) POPC/DSPC. Circles indicate experimental data. Solid lines define phase diagrams after correction for the finite width of the phase transitions of the pure components [4]. The dashed lines are not observed but assumed.

* T_o and T_f have been taken as the temperatures at which the calorimetric trace leaves and returns to baseline, after Blume and Ackermann [2]. In our opinion, this procedure provides the most accurate representation of the real width of a broad phase transition and is superior to approaches based on extrapolation of slopes. This is a point which should be carefully considered when comparing results from different laboratories, since data obtained by extrapolation techniques (including TEMPO spin label partitioning data) will always give transition widths which are smaller than those obtained by the procedure which we have used.

and 100% DMPC. The observed calorimetric behavior is summarized in Fig. 2a, in which we have plotted the onset (T_o) and completion (T_f) temperatures for each transition as a function of DMPC content *. Both T_o and T_f increase with increasing DMPC content over the entire compositional range. A thermal pretransition with the range 2–8°C was observed at 90% DMPC; samples at higher DMPC contents were not studied. Mixtures of POPC and DPPC were similarly studied; T_o and T_f are plotted as a function of DPPC content in Fig. 2b. The onset temperature remains constant over the compositional range 0–75% DPPC and increases over the range 75–100% DPPC. As the DPPC content is raised from 50 to 75%, it becomes increasingly difficult to assign T_o from heating runs. However, cooling runs at 2.5 deg. C/min clearly demonstrate that T_o remains invariant over this range. T_f increases with increasing DPPC content and levels off at high DPPC content. Finally, we have studied the thermal behavior of mixtures of POPC and DSPC and present T_o and T_f as a function of DSPC content in Fig. 2c. The transition onset temperature remains constant over the range 0–75% DSPC and increases over the range 75–100% DSPC. The transition completion temperature increases with increasing DSPC content and levels off at high DSPC content. A thermal pretransition was not observed for any POPC/DPPC or POPC/DSPC mixture studied. Mixtures at DPPC or DSPC contents greater than 90% were not studied.

The transition enthalpies (ΔH) have been measured for all lipid mixtures studied and are presented in Fig. 3. For POPC/DMPC mixtures, ΔH is constant over the entire compositional range (Fig. 3a). For POPC/DPPC mixtures, ΔH increases with increasing DPPC up to approx. 75% DPPC and then levels off (Fig. 3b). The behavior of ΔH for POPC/DSPC mixtures is similar to that observed for POPC/DPPC mixtures (Fig. 3c).

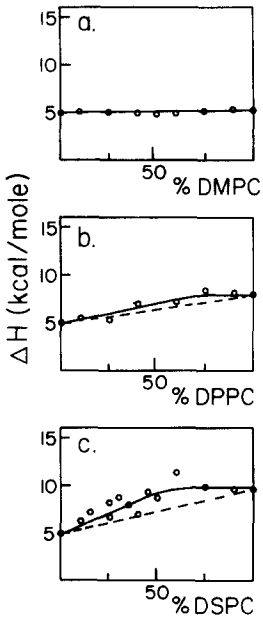

Fig. 3. Transition enthalpy as a function of lipid composition for (a) POPC/DMPC, (b) POPC/DPPC and (c) POPC/DSPC. Dashed line indicates ideal behavior.

^{2}H-NMR spectroscopy

^{2}H-NMR spectroscopy can be used to study the microscopic behavior of lipid mixtures in which either of the components is selectively deuterated [19,20]. This approach is particularly advantageous because the measured quadrupole splitting ($\Delta\nu_Q$) of the deuterium powder pattern is directly related to the orientational order of the C-^{2}H bond:

$$\Delta\nu_Q = \left(\tfrac{3}{4}\right)\left(e^2qQ/h\right)S_{CD}$$

where S_{CD} is the order parameter of the C-^{2}H bond and e^2qQ/h is the static quadrupole coupling constant (for a review, see Seelig [21]). Furthermore, the ability to label with deuterium at a variety of positions eliminates potential problems of interpretation due to local effects related to the position of the probe in the bilayer. Finally, the deuterium probe is essentially an isomorphic replacement for hydrogen, thus avoiding probe-generated perturbations of the system under study.

^{2}H-NMR spectroscopy was used to study the behavior of multilamellar dispersions of POPC, DPPC or POPC/DPPC in which either POPC or DPPC was deuterium-labeled in the 2-, 10- or 16-position of the acyl chain(s) or in the *N*-methyls of the choline head group. This approach was undertaken in order to gain some understanding of the behavior of the individual lipids in mixed POPC/DPPC bilayers, both in the liquid crystalline state and within the temperature range of the broad mixed-lipid phase transitions defined by DSC (Figs. 1 and 2).

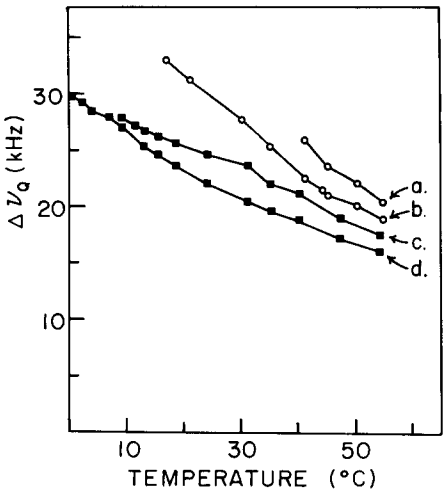

Fig. 4. Deuterium quadrupole splitting $\Delta\nu_Q$ vs. temperature for (a) [10,10′-$^2H_4$]DPPC, (b) 1:1 POPC/[10,10′-$^2H_4$]DPPC, (c) 1:1 [10–$^2H_2$]POPC/DPPC and (d) [10-$^2H_2$]POPC.

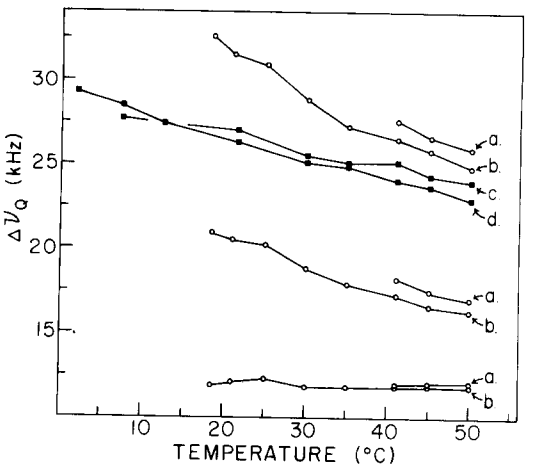

Fig. 5. Deuterium quadrupole splitting $\Delta\nu_Q$ vs. temperature for (a) [2,2′-$^2H_4$]DPPC, (b) 1:1 POPC/[2,2′-$^2H_4$]DPPC, (c) 1:1 [2-$^2H_2$]POPC/DPPC and (d) [2-$^2H_2$]POPC. ■, samples in which POPC is deuterated; ○, samples in which DPPC is deuterated. Samples in which POPC is deuterated exhibit only one set of quadrupole splittings, while those in which DPPC is deuterated exhibit three separate quadrupole splittings (see text for explanation).

The temperature dependence of $\Delta\nu_Q$ for [10-$^2H_2$]POPC,[10,10′-$^2H_4$]DPPC, 1 : 1 [10-$^2H_2$]POPC/DPPC and 1 : 1 POPC/[10,10′-$^2H_4$]DPPC is presented in Fig. 4. In the liquid crystalline state (e.g., 45°C), the magnitude of $\Delta\nu_Q$ increases in the order [10-$^2H_2$]POPC < 1 : 1[10-$^2H_2$]POPC/DPPC < 1 : 1 POPC/[10,10′-$^2H_4$]-DPPC < [10,10′-$^2H_4$]DPPC. On cooling in the liquid crystalline state, $\Delta\nu_Q$ for pure [10,10′-$^2H_4$]DPPC increases until 42°C (T_m for DPPC). At lower temperatures, the spectrum is no longer detectable due to the spectral width limitations of the spectrometer used. [10-$^2H_2$]POPC alone also exhibits an increase in $\Delta\nu_Q$ with decreasing temperature. In the 1:1 POPC/DPPC mixtures, the $\Delta\nu_Q$ for the 10-position of DPPC is greater than that of POPC at all temperatures studied. Furthermore, the difference in $\Delta\nu_Q$ between POPC and DPPC in the 1 : 1 mixture increases as the temperature is decreased through the broad phase transition defined by DSC. Note that the $\Delta\nu_Q$ values shown are for the residual liquid crystalline lipid. As the temperature is decreased, the intensity (signal/noise) of the signals from the residual liquid crystalline POPC and DPPC in the mixture decreases, with the intensity of the 1 : 1 POPC/[10,10′-$^2H_4$]DPPC signal decreasing more rapidly with temperature than that of 1:1 [10-$^2H_2$]POPC/DPPC (not shown). The signal from 1 : 1 POPC/[10,10′-$^2H_4$]DPPC is no longer detectable below approx. 17°C, while the signal from 1 : 1 [10-$^2H_2$]POPC/DPPC is detectable down to approx. 9°C.

In Fig. 5 is presented the temperature dependence of $\Delta\nu_Q$ for [2-$^2H_2$]POPC, [2,2′-$^2H_4$]DPPC, 1:1 [2-$^2H_2$]POPC/DPPC and 1:1 POPC/[2,2′-$^2H_4$]DPPC. Three separate doublets are observed from [2,2′-$^2H_4$]DPPC, as previously described by Seelig and Seelig [22]; thus, three $\Delta\nu_Q$ values are reported in Fig. 5 for those samples in which the deuterium label is in DPPC. The largest splitting has been shown by these authors to derive from the palmitoyl chain attached to the 1-position of the glycerol backbone, and the two smaller splittings are from the palmitoyl chain attached to the glycerol 2-position. The two sets of signals from the 2-chain result from magnetic non-equivalence of the two deuterons [23]. Only one set of splittings is observed for [2-$^2H_2$]POPC, as expected, since only the palmitoyl chain (attached to the glycerol *sn*-1 position) is labeled. Fig. 5 demonstrates that in the liquid crystalline state (e.g., 50°C), the magnitude of $\Delta\nu_Q$ for the [2-$^2H_2$]palmitoyl chain(s) increases in the order [2-$^2H_2$]POPC < 1 : 1 [$^2H_2$]POPC/DPPC < 1 : 1 POPC/[2,2′-$^2H_4$]DPPC < [2,2′-$^2H_4$]DPPC. As the temperature is decreased through the broad phase

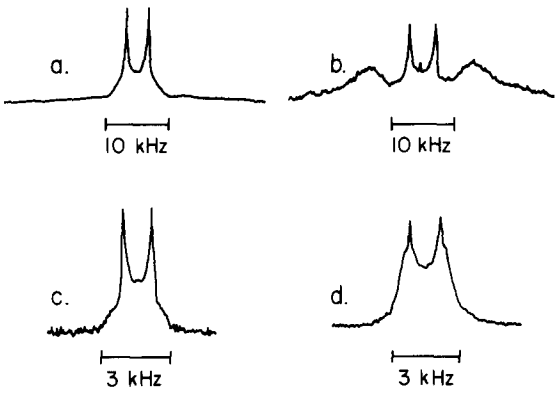

Fig. 6. Deuterium NMR spectra of (a) 1 : 1 [16-$^2H_3$]POPC/DPPC, (b) 1:1 POPC/[16,16′-$^2H_6$]DPPC, (c) 1:1 [*N-Me*-$^2H_9$]POPC/DPPC and (d) 1 : 1 POPC/[*N-Me*-$^2H_9$]DPPC. Spectra were obtained at 22°C, which is within the temperature range of the broad acyl chain order-disorder transition of 1:1 POPC/DPPC.

transition region of the mixture defined by DSC, the difference in $\Delta\nu_Q$ between the residual liquid crystalline POPC and DPPC increases, as was also observed for POPC/DPPC mixtures with the label in the acyl chain 10-position.

Similar observations have been made on POPC/DPPC mixtures labeled in the 16-position of the palmitoyl chain(s). In this case, the label is located at a chain position which is highly mobile so that the gel state spectrum can be observed with our spectrometer. In Fig. 6a, b are presented spectra of 1 : 1 [16-$^2H_3$]POPC/ DPPC and 1 : 1 POPC/[16,16′-$^2H_6$]DPPC at 22°C, a temperature within the broad transition range of the 1 : 1 POPC/DPPC mixture. A single liquid crystalline powder pattern is observed for the 16-labeled POPC in the 1 : 1 mixture (Fig. 6a), while an additional broad doublet characteristic of the gel state is observed for the 16-labeled DPPC in the mixture (Fig. 6b). Finally, we have obtained spectra of phospholipid mixtures labeled in the *N*-methyls of the choline head group. In Fig. 6c, d are presented spectra of 1 : 1 [*N-Me*-$^2H_9$]POPC/DPPC and 1 : 1 POPC/[*N-Me*-$^2H_9$]DPPC at 22°C. At this temperature, the DPPC in the 1 : 1 mixture exhibits a spectrum which is a superposition of gel state and liquid crystalline spectra (Fig. 6d). (In the case of *N-Me*-labeled phosphatidylcholines, the liquid crystalline and gel state spectra have similar quadrupole splittings; thus, a superposition of the two appears like a broadened spectrum with relatively sharp singularities.) At the same temperature, the POPC in the 1 : 1 mixture (Fig. 6c) appears to be almost entirely liquid crystalline.

Discussion

Equilibrium phase diagrams for POPC/DMPC, POPC/DPPC and POPC/DSPC have been derived from the calorimetric data after correction for the finite widths of the transitions of the pure components [4]. Because lipid phase transitions are not truly isothermal, such corrections are necessary to obtain phase diagrams, which are essentially idealizations of the experimental data [10]. POPC and DMPC exhibit complete but non-ideal miscibility in both gel and liquid crystalline states over the entire compositional range (Fig. 2a). The phase diagrams for the POPC/DPPC and POPC/DSPC systems both exhibit a horizontal solidus, indicating gel phase immiscibility over a large compositional range (Fig. 2b, c).

A relatively large number of binary phosphatidylcholine systems have been studied to date, and it is of interest to compare these with the present work in an attempt to derive some general conclusions. In Table I, we have summarized the published literature on miscibility in phosphatidylcholine mixtures, including the present work. The various binary systems have been arranged according to the absolute difference in temperature (ΔT_m) between the T_m values of the two pure components of each system. Binary systems for which $\Delta T_m < 33$°C exhibit gel state miscibility *, while those for which $\Delta T_m > 33$°C exhibit gel state immiscibility. It appears that, in general, phosphatidylcholines behave similarly in binary mixtures regardless of whether they are saturated, unsaturated or mixed-chain varieties.

Liquid crystalline immiscibility has not been observed in any of the PC-PC systems summarized in Table I. We conclude that acyl chain differences alone are not sufficient to cause lateral phase separations in the liquid crystalline state. If such

* There is disagreement on the gel state miscibility of mixtures of dielaidoylPC(18:1*t*/18:1*t*-PC) and dimyristoylPC (14:0/14:0-PC), as shown in Table I.

TABLE I

GEL STATE MISCIBILITY IN BINARY PHOSPHATIDYLCHOLINE MIXTURES

	T_m values (°C)	ΔT_m (deg. C)	Gel state miscibility	Ref.
18:0/16:0-PC, 16:0/18:0-PC	44,49	5	Yes	30
18:0/14:0-PC, 14:0/18:0-PC	30,39	9	Yes	30
16:0/14:0-PC, 14:0/16:0-PC	28,35	7	Yes	30
18:1*t*/18:1*t*-PC, 14:0/14:0-PC	12,23	11	No	3
18:1*t*/18:1*t*-PC, 14:0/14:0-PC	12,23	11	Yes	34
16:0/16:0-PC, 18:0/18:0-PC	42,55	13	Yes	1
14:0/14:0-PC, 16:0/16:0-PC	23,42	19	Yes	4
16:0/18:1*c*-PC, 14:0/14:0-PC	−3,23	26	Yes	a
18:1*t*/18:1*t*-PC, 16:0/16:0-PC	12,42	30	Yes	3
14:0/14:0-PC, 18:0/18:0-PC	23,55	33	Yes	1,4,31,32
18:0/18:1*c*-PC, 16:0/16:0-PC	9,42	33	No	33
14:0/14:0-PC, 20:0/20:0-PC	23,63 [b]	40	No	31
12:0/12:0-PC, 16:0/16:0-PC	−1,42	43	No	34
18:1*t*/18:1*t*-PC, 18:0/18:0-PC	12,55	43	No	3
18:1*c*/18:1*c*-PC, 14:0/14:0-PC	−20,23	43	No	7
16:0/18:1*c*-PC, 16:0/16:0-PC	−3,42	45	No	[a],33
12:0/12:0-PC, 18:0/18:0-PC	0,55	55	No	4
16:0/18:1*c*-PC, 18:0/18:0-PC	−3,55	58	No	[a]
18:1*c*/18:1*c*-PC, 16:0/16:0-PC	−20,42	62	No	7,9
18:1*c*/18:1*c*-PC, 18:0/18:0-PC	−20,55	75	No	7
18:1*c*/18:1*c*-PC, 22:0/22:0-PC	−20,75	95	No	7

[a] This work.
[b] Estimated.

interactions occur in natural membranes, they must be mediated by forces other than interchain van der Waal's interactions. Likely possibilities are sphingolipid clustering via intermolecular hydrogen bonding [13, 24–27] and cross-bridging of anionic lipids by divalent cations [28].

For the POPC/DMPC system, the constant enthalpy as a function of DMPC content indicates that POPC and DMPC mix nearly ideally (Fig. 3a). The composition dependences of the enthalpies for POPC/DPPC and POPC/DSPC exhibit deviations from ideality (Fig. 3b, c). This effect may be due to the heat of mixing on going from the phase-separated gel state to the mixed liquid crystalline state. The magnitude of this heat of mixing cannot be easily directly measured. Another possible origin for the larger observed enthalpy in these mixed-lipid systems is increased disorder in the liquid crystalline state relative to the liquid crystalline states of the pure components. A third possibility is increased order in the mixed-lipid gel state, compared to the pure components. We consider this unlikely and, in fact, would expect the mixed-lipid gel state to exhibit less acyl chain order than either of the pure components. However, this third possibility cannot be eliminated a priori. ^{2}H-NMR studies of the POPC/DPPC system, to be discussed below, indicate that POPC/DPPC mixtures in the liquid crystalline state are more disordered than POPC or DPPC alone at an equivalent reduced temperature. Some portion of the observed excess enthalpy in POPC/DPPC mixtures can be accounted for by this increased liquid crystalline disorder.

The ^{2}H-NMR studies of the POPC/DPPC system provide some interesting insights into the microscopic behavior of the two lipids in mixed bilayers. When compared at a single temperature in the liquid crystalline state, the average order of POPC/DPPC mixtures is intermediate between that of POPC and DPPC alone. This is demonstrated in Fig. 7a, b, c, d in which we have plotted $\Delta\nu_Q$ vs. percent DPPC at 50°C for the 2-, 10- and 16-positions of the acyl chain(s) and for the *N*-methyls of the choline head group. To a first approximation, lipid order appears to be linearly

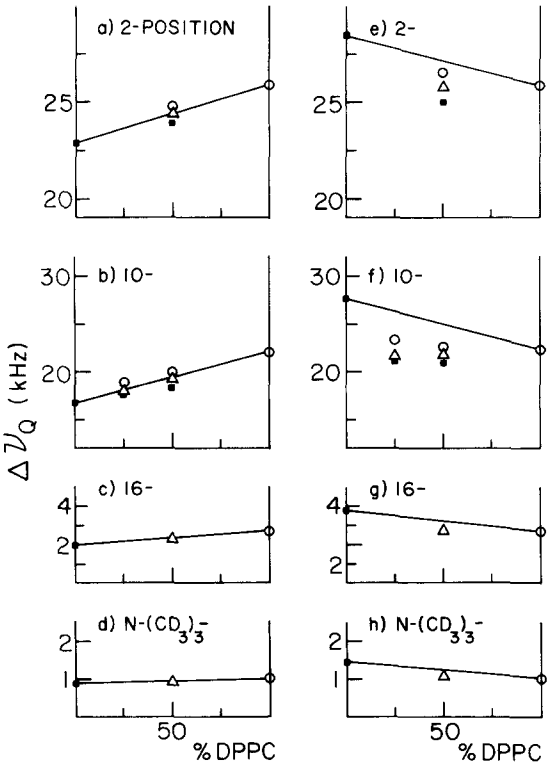

Fig. 7. Dependence of $\Delta\nu_Q$ on percent DPPC at four different label positions and at two temperatures (50°C and a reduced temperature in the liquid crystalline state). a and e present data for samples which are deuterium-labeled in the acyl chain 2-position; b and f have the label in the 10-position; c and g have the label in the 16-position; d and h have the label in the *N*-methyls of the choline head group. ■, $\Delta\nu_Q$ values from samples in which POPC was deuterium-labeled. ○, $\Delta\nu_Q$ values from samples in which DPPC was labeled. Δ, the average $\Delta\nu_Q$ for POPC/DPPC mixtures, i.e., the weighted average of the observed splittings of POPC and DPPC in each mixture. a–d demonstrate behavior at a single temperature in the liquid crystalline state, 50°C. e–h demonstrate behavior at a reduced temperature ($T_r = 0.0162$), with respect to the completion temperature of the cooperative phase transition observed by DSC (see text). The actual temperatures for which data are plotted in e–h are: 7.5°C for 0% DPPC (pure POPC), 32.7°C for 25% DPPC, 40.3°C for 50% DPPC and 49.3°C for 100% DPPC. In panels a and e, only one quadrupole splitting is shown for samples in which the 2-position of DPPC is labeled; this is the splitting from the DPPC *sn*-1 chain, which is comparable to the *sn*-1 palmitoyl chain of POPC.

related to acyl chain composition in the liquid crystalline state. Fig. 7a, b, c also shows that in POPC/DPPC mixtures, acyl chain $\Delta\nu_Q$ values are larger for DPPC than for POPC. Thus, the order of the palmitoyl chain(s) of POPC or DPPC is determined not only by the average order in the bilayer but also by the identity of the other chain in the deuterated phospholipid. This can be partially explained by noting that in the liquid crystalline phase, each acyl chain is surrounded in the bilayer plane by six acyl chains in an approximately hexagonal lattice. The deuterated 1-palmitoyl chain of [10-$^2H_2$]POPC, for example, is constrained by direct bonding to have an oleyl chain among its nearest neighbors, while the other five positions may be random. Either deuterated palmitoyl chain of [10,10′-$^2H_4$]DPPC is similarly constrained to have one directly bonded palmitoyl chain among its nearest neighbors.

It is instructive to compare the mixed and pure lipid systems at a reduced temperature in the liquid crystalline state. In Fig. 7e, f, g, h, we present the dependence of $\Delta\nu_Q$ on percent DPPC at the reduced temperature $T_r = (T - T_c)/T_c) = 0.0162$, where T_c is the completion temperature in K of the phase transition determined by DSC. The order of pure POPC is greater than that of pure DPPC at all acyl chain positions studied (as previously reported by Seelig and Seelig [29]) and at the choline head group. Measured at the 10-position (Fig. 7f), the average $\Delta\nu_Q$ is clearly smaller for POPC/DPPC mixtures than expected on the basis of a linear combination of the $\Delta\nu_Q$ values of the pure lipids at the same reduced temperature. Similar results are observed at the acyl chain 2- and 16-positions (Fig. 7e, g) and at the choline head group (Fig. 7h). These results indicate that POPC/DPPC mixtures in the liquid crystalline state are more disordered than POPC or DPPC alone at an equivalent reduced temperature. This observation is consistent with enthalpy measurements for the POPC/DPPC system, which indicate that the enthalpy of the order-disorder transition of POPC/DPPC mixtures is greater than predicted from the enthalpies of the pure lipids alone.

It should be emphasized that, although comparison of lipid order at a reduced temperature is relevant from a physical chemical point of view, biological membranes essentially exist in an isothermal world. Although POPC is more ordered than DPPC when compared at a reduced temperature, POPC is more disordered than DPPC under the biologically relevant isothermal condition.

When observed at a single temperature significantly above the phase transition of the pure components, addition of DPPC to POPC results in increased order in an essentially linear fashion.

As the temperature of a 1 : 1 POPC/DPPC mixture is decreased into the broad phase transition region, a number of interesting changes occur. In the liquid crystalline state (greater than 35°C), the $\Delta\nu_Q$ values for 1 : 1 [10-$^2H_2$]POPC/DPPC and 1 : 1 POPC/[10,10′-$^2H_4$]DPPC exhibit similar temperature dependences (Fig. 4). As the samples are cooled further into the phase transition region defined by DSC (−4 to 35°C), the difference in $\Delta\nu_Q$ between 1 : 1 [10-$^2H_2$]POPC/DPPC and 1 : 1 POPC/[10,10′-$^2H_4$]DPPC increases. (Note that these $\Delta\nu_Q$ values are for the residual liquid crystalline lipid, since we cannot observe gel state lipid at this label position.) Finally, at lower temperatures, $\Delta\nu_Q$ for 1 : 1 [10-$^2H_2$]POPC/DPPC approaches that for pure [10-$^2H_2$]POPC. Thus, as the temperature is decreased through the broad 1 : 1 POPC/DPPC phase transition, mixing of the liquid crystalline POPC and DPPC becomes highly non-ideal. Furthermore, as the temperature is decreased through the broad phase transition, the NMR signal-to-noise ratio decreases for both the residual liquid crystalline 1 : 1 [10-$^2H_2$]-POPC/DPPC and 1 : 1 POPC/[10,10′-$^2H_4$]-DPPC, as more of each lipid enters the gel state (not shown). The signal for 1 : 1 POPC/[10,10′-$^2H_4$]DPPC is not detectable at temperatures below approx. 17°C, while that for 1 : 1 [10-$^2H_2$]POPC/DPPC is detectable down to approx. 9°C. This is consistent with progressive enrichment of the liquid crystalline phase with POPC as the temperature is lowered *. Studies carried out on 1 : 1 [2-$^2H_2$]-POPC/DPPC and 1 : 1 POPC/[2,2′-$^2H_4$]DPPC provide similar results (Fig. 5) and point to identical conclusions. Spectra of 1 : 1 [16-$^2H_3$]POPC/DPPC and 1 : 1 POPC/[16,16′-$^2H_6$]DPPC clearly indicate that, within the temperature range of the broad phase transition, the gel phase is DPPC-rich and the liquid crystalline phase is POPC-rich.

In conclusion, we have shown that, in binary mixtures, the naturally occurring phosphatidylcholine POPC exhibits miscibility characteristics which are similar to those exhibited by the well-studied disaturated phosphatidylcholines. Our ^{2}H-NMR results clearly demonstrate that within the temperature range of broad mixed lipid phase transitions, the component lipids are highly demixed.

* It should be noted that virtually any binary system will exhibit progressive enrichment of the liquid crystalline phase in the lower melting component as the temperature is decreased, with the magnitude of the effect determined by the shape of the phase diagram. This is not evidence for gel state immiscibility, which is defined by independence of the transition onset temperature with composition.

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