Bilayer Interactions of Ether- and Ester-Linked Phospholipids: Dihexadecyl- and Dipalmitoylphosphatidylcholines†

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ABSTRACT: Mixed phospholipid systems of ether-linked 1,2-dihexadecylphosphatidylcholine (DHPC) and ester-linked 1,2-dipalmitoylphosphatidylcholine (DPPC) have been studied by differential scanning calorimetry and X-ray diffraction. At maximum hydration (60 wt % water), DHPC shows three reversible transitions: a main (chain melting) transition, T_M = 44.2 °C; a pretransition, T_P = 36.2 °C; and a subtransition, T_S = 5.5 °C. DPPC shows two reversible transitions: T_M = 41.3 °C and T_P = 36.5 °C. T_M decreases linearly from 44.2 to 41.3 °C as DPPC is incorporated into DHPC bilayers; T_P exhibits eutectic behavior, decreasing sharply to reach 23.3 °C at 40.4 mol % DPPC and then increasing over the range 40–100 mol % DPPC; T_S remains constant at 4–5 °C and is not observed at >20 mol % DPPC. At 50 °C, X-ray diffraction shows a liquid-crystalline bilayer L_α phase at all DHPC:DPPC mole ratios. At 22 °C, DHPC shows an interdigitated bilayer gel L_β phase (bilayer periodicity d = 47.0 Å) into which ~30 mol % DPPC can be incorporated. Above 30 mol % DPPC, a noninterdigitated gel $L_{\beta'}$ phase (d = 64–66 Å) is observed. Thus, at $T > T_M$, DHPC and DPPC are miscible in all proportions in an L_α bilayer phase. In contrast, a composition-dependent gel → gel transition between interdigitated and noninterdigitated bilayers is observed at $T < T_P$, and this leads to eutectic behavior of the DHPC/DPPC system.

For membrane phospholipids, attention is now being paid to the mode(s) of attachment of the hydrocarbon chains to the glycerol moiety. The usual linkage is through an ester bond (acyl), but ether (alkyl) and vinyl ether (alk-1-enyl) linkages are found and in some cases (e.g., platelet activating factor) contribute to the functional potency of lipids [for a recent review, see Snyder (1985)]. Recently, the effects of chain linkage on phospholipid structure and properties have begun to be studied. For example, the bilayer structure and properties of diacyl- and dialkylphosphatidylcholines have been compared (Vaughan & Keough, 1974; Ruocco et al., 1985a,b; Kim et al., 1987). While the thermotropic properties of hydrated 1,2-dihexadecylphosphatidylcholine (DHPC) resemble those of its ester analogue, 1,2-dipalmitoylphosphatidylcholine (DPPC), marked structural and dynamic differences between DHPC and DPPC are found (Siminovitch et al., 1983; Ruocco et al., 1985a,b; Kim et al., 1987). Most striking is the observation that fully hydrated DPPC forms only conventional bilayer phases (L_c, $L_{\beta'}$, $P_{\beta'}$, and L_α), whereas hydrated DHPC can exist either in a conventional bilayer gel phase ($L_{\beta'}$) at low hydration (Kim et al., 1987) or in a chain-interdigitated (L_β) gel phase at high hydration (Ruocco et al., 1985a; Kim et al., 1987). Thus, altered chain linkage alone can produce significant changes in bilayer structure and chain packing arrangements.

Recognizing these structural differences between DHPC and DPPC, we have now examined their miscibility in gel and liquid-crystalline bilayer phases, focusing on the ability of DHPC to incorporate into conventional bilayers and, conversely, the ability of DPPC to partition into chain-interdigitated bilayers.

Materials and Methods

DHPC (Berchtold, Berne, Switzerland) and DPPC (Avanti, Birmingham, AL) were purified by column chromatography and shown to be >99% pure by thin-layer chromatography using the solvent system chloroform/methanol/water (65:25:4 v/v).

For differential scanning calorimetry (DSC), samples of anhydrous DHPC and DPPC were weighed into stainless steel DSC pans to give different DHPC:DPPC mole ratios and dissolved in chloroform. Chloroform was removed initially under nitrogen and then overnight under vacuum. Distilled, deionized water was added gravimetrically (~60 wt %), and the pans were hermetically sealed. For X-ray diffraction, a similar mixing procedure to that described above was used except that the samples were prepared in constricted glass tubes and after equilibration at 50 °C transferred to thin-walled glass capillaries. The capillaries were flame-sealed and coated at the seal with epoxy glue to eliminate leaks.

DSC and X-ray diffraction measurements were made as described in the preceding paper (Kim et al., 1987). X-ray diffraction data were also recorded with a position-sensitive linear detector. Nickel-filtered Cu Kα radiation from a microfocus X-ray generator (Jarrell-Ash, Waltham, MA) was line-focused by a single mirror and collimated with the slit optical system of a Luzzati–Baro camera. Intensity data were recorded by using a linear position-sensitive detector (Tennelec, Oak Ridge, TN) and associated electronics (Tracor Northern, Middleton, WI).

Results

DSC of Fully Hydrated DHPC/DPPC Bilayers. DSC of fully hydrated DHPC exhibits reversible transitions at 5.5, 36.2, and 44.2 °C corresponding to subtransitions (T_S), pretransitions (T_P), and chain melting transitions (T_M), respectively [Figure 1A; see also Ruocco et al. (1985a) and Kim et al. (1987)]. With increasing mole percent DPPC, similar thermotropic behavior is observed with the exception that at >20 mol % DPPC no subtransition is observed (for example, see Figure 1A). T_M decreases with increasing mole percent DPPC in an approximately linear fashion (Figure 1B); however, T_P exhibits more complex behavior. T_P decreases in temperature, reaching a minimum value (T_P = 22 °C) at ~42

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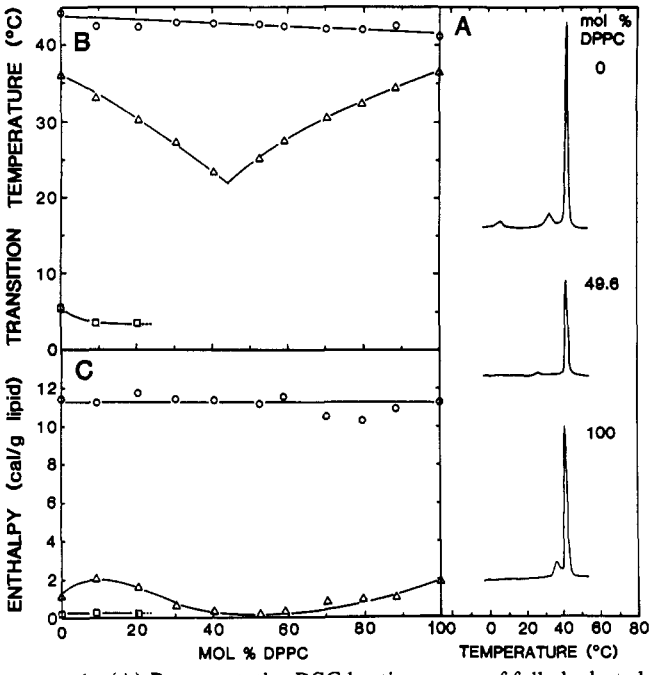

FIGURE 1: (A) Representative DSC heating curves of fully hydrated (60–70 wt % water) DHPC, DHPC/DPPC (49.6 mol % DPPC), and DPPC. (B) Transition temperature (degrees centigrade) and (C) transition enthalpy (calories per gram of lipid) as a function of mole percent DPPC for fully hydrated DHPC/DPPC mixtures. (○) T_M; (△) T_P; (□) T_S.

mol % DPPC; at >42 mol % DPPC, T_P progressively increases in temperature, reaching the limiting value (T_P = 36.5 °C) characteristic of DPPC (Figure 1B). The corresponding transition enthalpies ΔH_S, ΔH_P, and ΔH_M (expressed as calories per gram of total lipid) are shown in Figure 1C. ΔH_S stays essentially constant at ~0.2 cal/g over the range 0–20 mol % DPPC. ΔH_P (1.3 cal/g for DHPC) exhibits a complex dependence on composition with a minimum value (~0.2 cal/g) at 40–50 mol % DPPC (Figure 1C). It should be noted, however, that for intermediate compositions T_P can be quite broad (see Figure 1) and accurate enthalpy determinations in this range are difficult to obtain. Finally, ΔH_M exhibits an approximately linear dependence on the DHPC:DPPC mole ratio (Figure 1C).

X-ray Diffraction of Fully Hydrated DHPC/DPPC Bilayers. Representative X-ray diffraction patterns of DHPC, DPPC, and DHPC/DPPC mixtures at 22 and 50 °C are shown in Figure 2A–J. At 22 °C, fully hydrated DHPC exhibits lamellar reflections (h = 1–5) corresponding to a bilayer periodicity d = 47.0 Å and a single, sharp wide-angle reflection at 1/4.08 Å^{-1} (Figure 2A). A similar diffraction pattern with a slightly increased periodicity (d = 48.9 Å) is observed at 19.3 mol % DPPC (Figure 2B). At 49.9 mol % DPPC, the diffraction pattern has changed significantly (Figure 2C): lamellar reflections (h = 1–5) corresponding to an increased bilayer periodicity, d = 65.5 Å, are observed, and the intensity distribution differs markedly from that observed at low DPPC content (cf. panels B and C of Figure 2). A single wide-angle reflection at 1/4.19 Å^{-1} is observed. At 77.3 mol % DPPC, a similar diffraction pattern is observed (Figure 2D) with a slightly reduced periodicity (d = 63.6 Å), and an additional broad wide-angle reflection at 1/3.96 Å^{-1} is present. This diffraction pattern is essentially identical with that observed for DPPC (d = 63.6 Å; Figure 2E). The bilayer periodicity is plotted as a function of mole percent DPPC in Figure 3A. At low mole percent DPPC, a single bilayer phase of small periodicity (d = 47–49 Å) is observed. At 28.7 mol % DPPC, two bilayer phases are observed with periodicities d = 68.0 and 48.5 Å. For >30 mol % DPPC, only a single bilayer phase is observed, the periodicity of which shows a small compositional dependence decreasing from 65.6 Å at 39% DPPC to 63.6 Å for DPPC alone.

Assuming that the mixed DHPC/DPPC bilayer phases of low periodicity (47–49 Å) observed at <30 mol % DPPC (T = 22 °C) are similar to those of DHPC [see Ruocco et al. (1985a) and Kim et al. (1987)], the lamellar structure amplitudes can be phased. For h = 1–5, the phase combination −, −, +, −, − is used to calculate the electron density profiles, $\rho(X)$, shown in Figure 4A (bottom). For mixed DHPC/DPPC bilayer phases of higher periodicity (d = 64–66 Å) observed at >30 mol % DPPC (T = 22 °C), the phasing demonstrated previously for DPPC (Torbet & Wilkins, 1976; McIntosh & Simon, 1986) is assumed (h = 1–5; −, −, +, −, −), and the resulting electron density profiles are also shown in Figure 4A (top). For <30 mol % DPPC, the electron density profiles show peaks at X = ±15 Å, corresponding to the location of the phosphate groups on either side of the bilayer, with no well-defined trough at the bilayer center (X = 0 Å). As shown elsewhere for many PC systems (Serrallach et al., 1983; McDaniel et al., 1983; McIntosh et al., 1983; Simon & McIntosh, 1984; Mattai & Shipley, 1986) including DHPC (Ruocco et al., 1985a; Kim et al., 1987), these features are characteristic of an interdigitated bilayer. In this structure, hydrocarbon chains from both sides of the "bilayer" interpenetrate with resultant reduction in the expected bilayer thickness. In contrast, at >30 mol % DPPC, the profiles show two electron-rich phosphate peaks at X = ±21–23 Å and a pronounced trough at the bilayer center. These profiles are characteristic of the usual bilayer arrangement, the two "monolayers" being separated by a region occupied by chain terminal methyl groups, and have been observed for a number of PC systems (Levine et al., 1968; Torbet & Wilkins, 1976; Janiak et al., 1979; McIntosh & Simon, 1986).

At 50 °C, similar lamellar X-ray diffraction patterns are observed for DHPC (d = 67.8 Å; Figure 2F), DPPC (d = 66.4 Å; Figure 2J), and all DHPC/DPPC mixtures (see Figure 2G–I). A single, broad wide-angle reflection at 1/4.5 Å^{-1} indicates melted hydrocarbon chains, and a liquid-crystalline L_α phase is present at all DHPC:DPPC mole ratios. The bilayer periodicity, d, plotted as a function of mole percent DPPC in Figure 3B, shows a small compositional dependence with a minimum value of d being observed at ~50 mol % DPPC. Again, structure amplitudes were assigned the phases (h = 1–4) −, −, +, − as shown previously for DHPC (Kim et al., 1987) and DPPC (G. G. Shipley, unpublished observations) in the liquid-crystalline state at full hydration. Corresponding electron density profiles calculated for DHPC, DPPC, and DHPC/DPPC mixtures are shown in Figure 4B. The profiles are typical of other liquid-crystalline PC bilayers (Torbet & Wilkins, 1976; McIntosh & Simon, 1986; Ruocco et al., 1985a; Kim et al., 1987) and are similar at all DHPC:DPPC ratios. The bilayer thickness is essentially independent of composition, d_{p-p} = 46 Å.

Discussion

Molecular interactions between different phospholipids have been studied in bilayer systems. In general, these systems exhibit miscibility in the melted chain states, although with the appropriate mixtures composition-dependent transitions between bilayer and nonbilayer (particularly hexagonal HII) structures can occur (Cullis et al., 1978; Goldfine et al., 1987). In contrast, below the chain melting transition, a number of different phase relationships can occur. For example, ideal

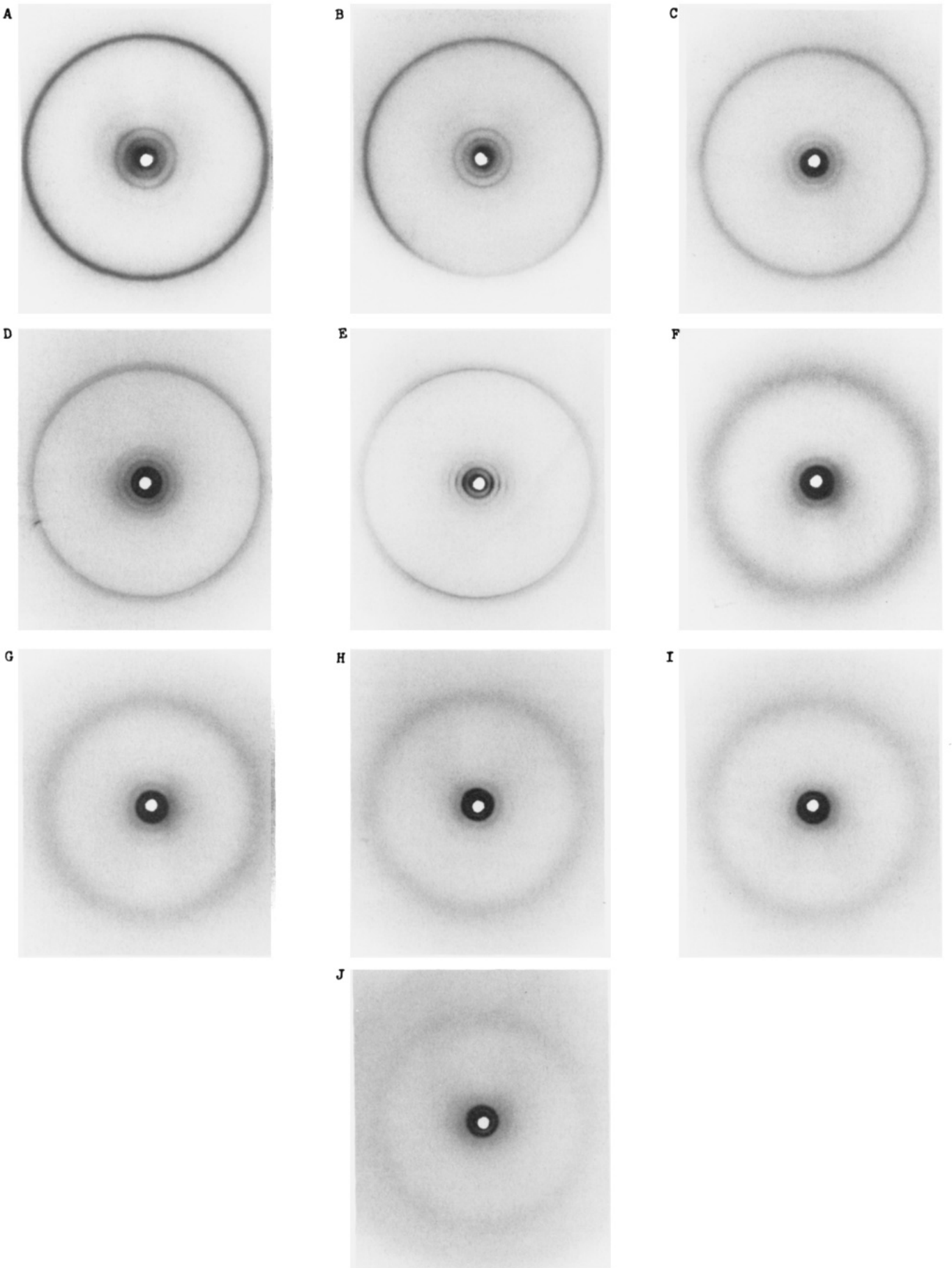

FIGURE 2: Representative X-ray diffraction patterns of fully hydrated DHPC/DPPC mixtures. (A) 100:0 DHPC:DPPC, 22 °C; (B) 80.7:19.3, 22 °C; (C) 50.1:49.9, 22 °C; (D) 22.7:77.3, 22 °C; (E) 0:100, 22 °C; (F) 100:0, 50 °C; (G) 80.7:19.3, 50 °C; (H) 50.1:49.9, 50 °C; (I) 22.7:77.3, 50 °C; (J) 0:100, 50 °C.

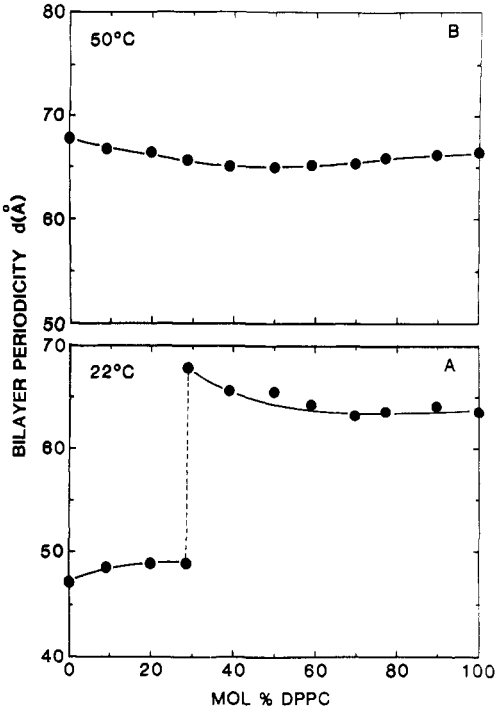

FIGURE 3: Bilayer periodicity, d (angstroms), as a function of mole percent DPPC for fully hydrated DHPC/DPPC mixtures at 22 °C (A) and 50 °C (B).

mixing (solid solution), phase separation, or eutectic or peritectic behavior can be observed at $T < T_M$ depending on the binary lipid system.

For binary systems of PCs with chain lengths in the range 10–18 carbon atoms, essentially ideal miscibility is observed in the L_α [but see Knoll et al. (1983)] and $P_{\beta'}$ phases, whereas miscibility in the gel state is dependent on the chain length difference of the two PC species (Phillips et al., 1970; Shimshick & McConnell, 1973; Mabrey & Sturtevant, 1976). If the chain length difference is <4 carbon atoms, gel phase miscibility is observed at all compositions. If the difference is >4 carbons, lateral phase separation of the higher melting (longer chain) PC occurs.

For the DHPC/DPPC binary system studied here, predictably, miscibility is observed in the fluid L_α phase (see Figure 5, top). However, below T_M, eutectic behavior is observed. In the region between T_P and T_M, the DHPC/DPPC mixtures exhibit solid solution behavior, with DHPC, DPPC, and all DHPC/DPPC mixtures forming the rippled bilayer gel phase defined structurally for DPPC [see Tardieu et al. (1973) and Janiak et al. (1976, 1979)]. Most striking is the compositional dependence of T_P. T_P decreases from 36.2 °C for DHPC to a minimum of ~22 °C at ~42 mol % DPPC and then increases to 36.5 °C for DPPC. Thus, the DHPC/DPPC system exhibits eutectic behavior with a eutectic point at ~22 °C and ~42 mol % DPPC. The eutectic behavior of the gel → gel transition T_P is unusual in PC systems. Eklund et al. (1984) report DSC data suggestive of eutectic behavior of T_P in mixtures of D and L enantiomers of dimyristoylphosphatidylcholine; however, the maximal depression in T_P is only 1.8 °C.

The basis of the eutectic behavior exhibited by T_P in the DHPC/DPPC system is the presence of two gel phases of different structure below T_P. At low mole percent DPPC, the interdigitated gel phase exhibited by DHPC can incorporate up to ~30 mol % DPPC (see Figure 5, bottom left). At >50 mol % DPPC, a noninterdigitated bilayer phase ($L_{\beta'}$) of the type exhibited by DPPC is present (see Figure 5, bottom right).

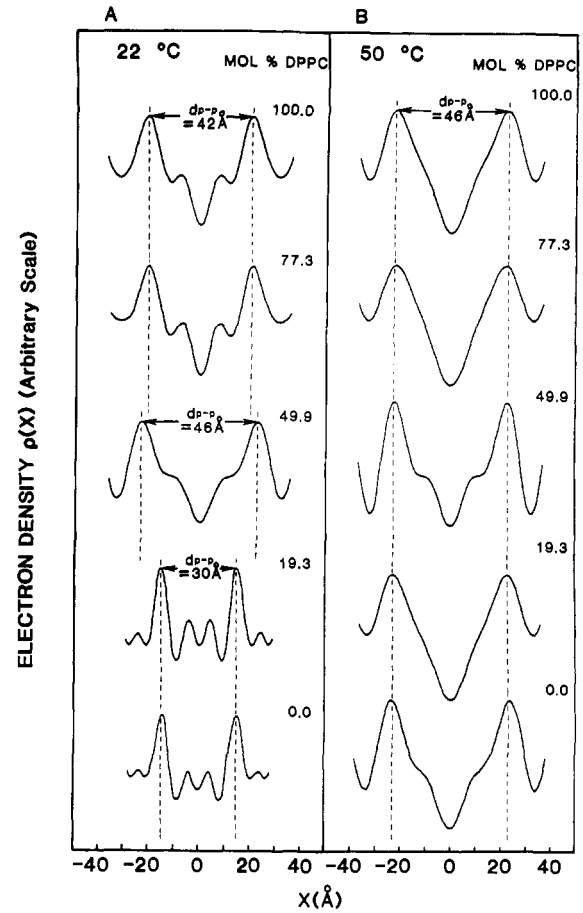

FIGURE 4: Representative electron density profiles of fully hydrated DHPC/DPPC bilayers (100:0, 80.7:19.3, 50.1:49.9, 22.7:77.3, and 0:100 mole ratios of DHPC:DPPC) at (A) 22 °C and (B) 50 °C. Intensity data were corrected for Lorentz and polarization factors and converted to normalized amplitudes (Worthington & Blaurock, 1969). For phase assignments, see text.

There is some evidence that increased DHPC content reduces the chain tilt in the $L_{\beta'}$ phase (see wide-angle diffraction data in Figure 2). Thus, DHPC is incorporated into this bilayer phase (at 22 °C, up to ~50 mol % DHPC). Between ~30 and 50 mol % DPPC (the actual phase boundaries are not clearly defined by the X-ray diffraction experiments), a two-phase region is present consisting of the interdigitated and noninterdigitated bilayer phases.[1] DHPC containing up to ~20 mol % DPPC exhibits a reversible subtransition at ~5 °C, below which a subgel phase is present. In this study, we have not considered the slowly reversible subtransition (and subgel, L_c phase) exhibited by DPPC (Chen et al., 1980; Füldner, 1981; Ruocco & Shipley, 1982a,b) and, perhaps, by DPPC-rich DHPC/DPPC mixtures. An idealized phase diagram describing the DHPC/DPPC system at full hydration is shown in Figure 5.

Finally, it is notable that DPPC which prefers the usual noninterdigitated bilayer gel phase can be incorporated up to ~30 mol % into the interdigitated bilayer phase favored by DHPC. It should, however, be pointed out that DPPC itself can be induced into an interdigitated phase by interaction with solute molecules [such as alcohols; see Simon and McIntosh (1984) and McDaniel et al. (1983)], surface-active drugs (McIntosh et al., 1983), or high pressures (Braganza & Worcester, 1986). Thus, the potential for DPPC forming

[1] Our X-ray diffraction studies recorded at a temperature (22 °C) close to the eutectic temperature do not allow us to define clearly the compositional limits of the phases present below the eutectic temperature (see legend to Figure 5).

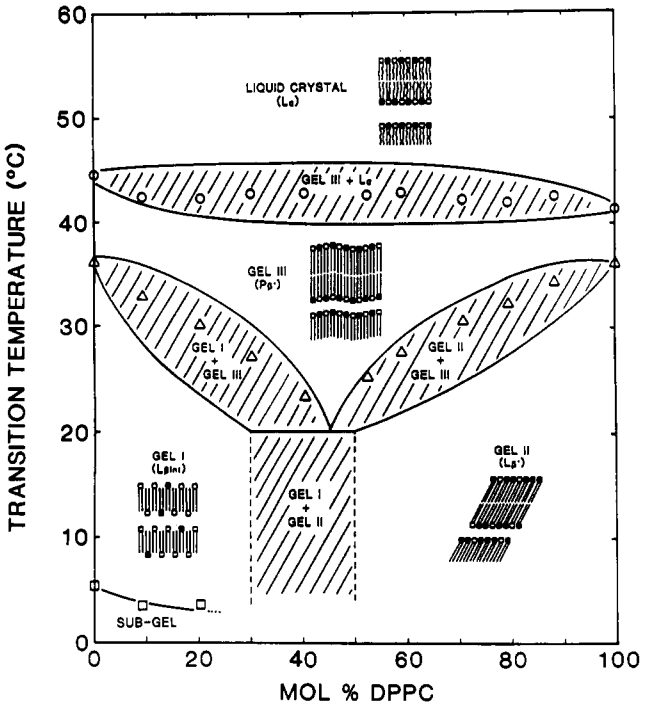

FIGURE 5: Idealized phase diagram of the DHPC/DPPC system based on the DSC and X-ray diffraction data. Onset and completion temperatures are not accurately determined from DSC experiments due to heating rate effects etc. In addition, the width and shape of the central two-phase region of coexisting gel phases have not been precisely determined. DHPC molecules are shown with open squares and DPPC molecules with closed squares representing the polar group.

interdigitated bilayer phases clearly exists. Conversely, DHPC which prefers to form an interdigitated bilayer phase [at least at high hydration; see Ruocco et al. (1985a) and Kim et al. (1987)] can be incorporated (up to ~50 mol %) into the noninterdigitated bilayer gel phase. Again, it should be stated that although DHPC prefers an interdigitated bilayer gel phase, a reduction in the degree of hydration is sufficient to convert it to a noninterdigitated bilayer phase (Kim et al., 1987). Thus, PCs can interconvert between noninterdigitated and interdigitated bilayers by simple alterations of the chemistry [cf. DPPC with DHPC [see Ruocco et al. (1985a)] and β-DPPC [see Serrallach et al. (1983)]], mixing different types of PCs (e.g., DPPC and DHPC; this study), alteration of the solvent [see Simon and McIntosh (1984) and McDaniel et al. (1983)], addition of surface-active molecules [see McIntosh et al. (1983)], or alteration in a thermodynamic variable [e.g., pressure; see Braganza and Worcester (1986)]. At present, this property seems to be particularly associated with PCs rather than other phospholipid classes and again emphasizes the "structural flexibility" of this particular membrane lipid.

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Registry No. DHPC, 18545-87-4; DPPC, 2644-64-6.

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