Deuterium Magnetic Resonance Study of Phase Equilibria and Membrane Thickness in Binary Phospholipid Mixed Bilayers†

M. B. Sankaram* and T. E. Thompson

Department of Biochemistry, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

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ABSTRACT: The gel-fluid phase equilibrium in a two-component system formed from dimyristoylphosphatidylcholine (DMPC) and distearoylphosphatidylcholine (DSPC) was investigated using solid-state wide-line ^2H NMR spectroscopy. Analysis of the spectral first moments and the quantitation of gel and fluid phases by means of difference spectroscopy provided the temperature–composition phase diagrams. Phase diagrams were constructed for mixtures of perdeuterated DMPC, DMPC-d_{54}, with DSPC and for the complementary system comprised of DMPC and perdeuterated DSPC, DSPC-d_{70}. The gel–fluid coexistence region was found to extend over a wider range of temperature and composition for the DMPC-d_{54}–DSPC system than for the DMPC–DSPC-d_{70} system. Comparison of these data with the phase diagram for the DMPC–DSPC system showed that in the gel–fluid region the fraction of lipids in the fluid phase at a given temperature and system composition decreases for the three systems in the order DMPC-d_{54}–DSPC > DMPC–DSPC > DMPC–DSPC-d_{70}. While the fluid fraction varies by as much as 90% among the three systems, the composition of the fluid phase, i.e., the ratio of the concentrations of the two molecules in the fluid phase, varies by about 20% over the whole temperature and system composition range. The effective acyl chain lengths of the DMPC-d_{54} and DSPC-d_{70} molecules as a function of temperature and composition in the fluid phase, when the system is all fluid or is in the gel–fluid coexistence region, were calculated from the quadrupole splittings in the axially symmetric powder patterns obtained for the all-fluid phase. The magnitudes of the coefficient of thermal expansion for both the DMPC-d_{54} and the DSPC-d_{70} molecules were smaller in the fluid phase of binary mixtures than in one-component bilayers containing either DSPC-d_{70} or DMPC-d_{54} alone. In addition, at any given temperature in the fluid phase, the increase in the acyl chain length of DMPC-d_{54} with increasing DSPC content of the system was smaller than the concomitant increase in the length of DSPC-d_{70} in mixtures with DMPC. In the entire temperature and composition range when the binary mixtures are in the all-fluid or in the gel–fluid coexistence region, the largest value obtained for the DMPC-d_{54} molecule in the fluid phase was smaller than the smallest value obtained for the DSPC-d_{70} molecule in the fluid phase. The acyl chain lengths were used to calculate the effective weighted-average thickness, d, of the fluid phase bilayer. The thickness was obtained for a time- and weighted-average configuration, which allows for a smooth bilayer surface but has a mismatch at the center of the bilayer that leads to a partially interdigitated structure. As expected, increasing the temperature at any given mole fraction of DSPC or DSPC-d_{70} resulted in a decrease in d. Whether the system is all-fluid or is in the gel–fluid coexistence region, the bilayer thickness of the fluid phase at any given temperature was found to increase nearly *linearly* with increasing mole fraction of DSPC or DSPC-d_{70} in the fluid phase.

The biological relevance of fundamental physical and chemical processes in model membrane systems has led to a major effort in studies of the influence of lipid composition on the global and local structural properties of membrane systems (Cevc & Marsh, 1987). Among the various studies on different model systems, investigations on bilayers formed from mixtures of phospholipids are expected to provide insights into the structure and dynamics of bilayers that have a direct impact on biological processes. This is because these systems are heterogeneous in chemical composition, exhibit phase separation, and form isolated in-plane lipid domains; features which are found in many plasma and intracellular membranes [see, e.g., Jain (1988), Vance and Vance (1985), and Thompson and Huang (1986)].

In this paper, we present results on a binary lipid mixture formed from dimyristoylphosphatidylcholine (DMPC)[1] and distearoylphosphatidylcholine (DSPC). This system exhibits the properties mentioned above (Mabrey & Sturtevant, 1976; Vaz et al., 1989). Of specific concern here is the effect on the bilayer thickness of mixing together the relatively shorter DMPC molecule and the long DSPC molecule. The mismatch in the lengths of the molecules may be annulled by an increase in the gauche conformer population for the DSPC molecule and a decrease for DMPC. Alternatively, the differences in lengths of the two molecules may persist when they are packed together to form a lipid bilayer of varying composition, but

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* Author to whom correspondence should be addressed.

[1] Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPC-d_{54}, 1,2-bis(perdeuteriomyristoyl)-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSPC-d_{70}, 1,2-bis-(perdeuteriostearoyl)-*sn*-3-glycero-3-phosphocholine; NMR, nuclear magnetic resonance.

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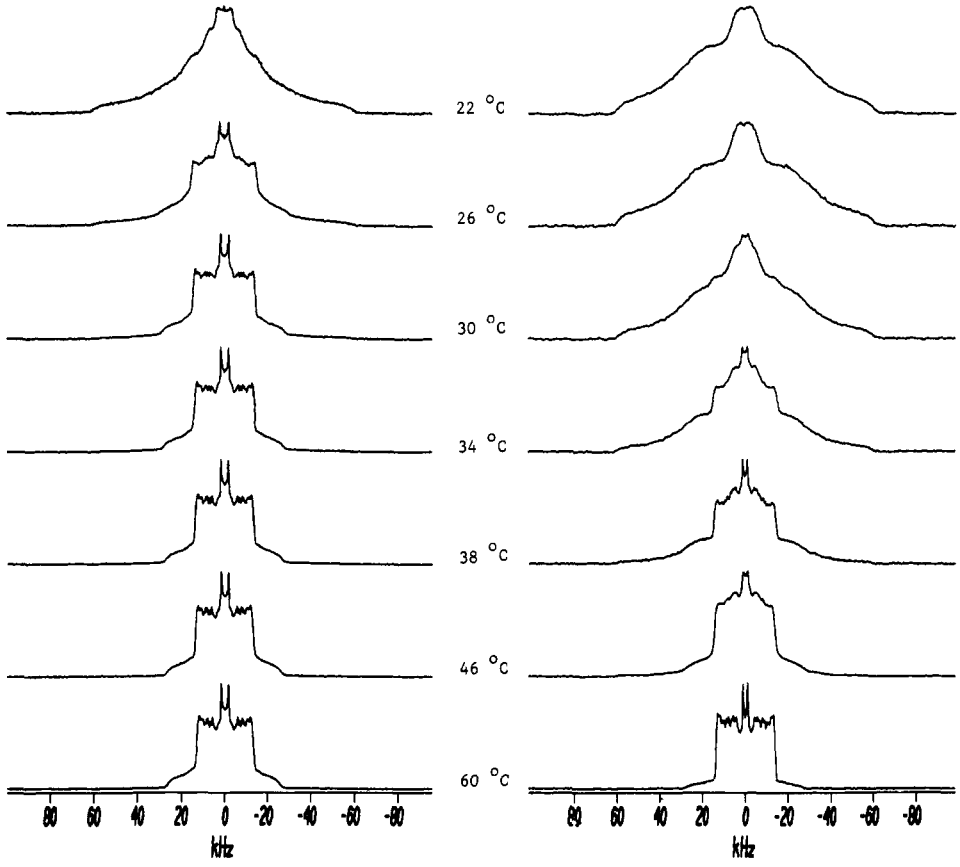

FIGURE 1: 55-MHz ^{2}H NMR spectra at different temperatures of perdeuterated phospholipids in equimolar binary mixtures. The left panel shows the NMR spectra at 22, 26, 30, 34, 38, 46, and 60 °C obtained from an equimolar mixture of perdeuterated dimyristoylphosphatidylcholine, DMPC-d_{54}, and distearoylphosphatidylcholine, DSPC. The right panel shows spectra at the same temperatures arising from the complementary equimolar mixture of dimyristoylphosphatidylcholine, DMPC, and perdeuterated distearoylphosphatidylcholine, DSPC-d_{70}.

the mode of packing can be altered from that known for conventional one-component bilayers so as to accommodate the lipids in a uniform bilayer. Knowledge of the effective acyl chain lengths of the two molecules in binary mixtures is required to distinguish between the two possibilities.

It is possible to obtain this information by using ^{2}H NMR spectroscopic methods on deuterium–labeled phospholipids (Seelig, 1980; Davis, 1979, 1983). The strategy employed is to perdeuterate one of the two lipids of this system and to monitor the spectral properties of that component as a function of temperature and as the concentration of the other component is varied. Under conditions where the system is in the gel–fluid coexistence region, one can monitor the spectra of the perdeuterated component in both the phases. The complementary set of experiments where the previously nonperdeuterated (protiated) component is now perdeuterated, and the other component is protiated, then provide the necessary data to determine the bilayer thickness of the fluid phase in both the one- and two-phase regions of the temperature–composition phase diagram. Both component molecules of the binary system give well-resolved axially symmetric spectra only for the fluid phase. Hence the present study deals with the effective acyl chain lengths and bilayer thickness only for the fluid phase, but under conditions when this is the only phase and when both fluid and gel phases coexist.

MATERIALS AND METHODS

Materials. Perdeuterated dimyristoyl phosphatidylcholine, DMPC-d_{54}, and perdeuterated distearoylphosphatidylcholine, DSPC-d_{70}, were purchased from Avanti Polar Lipids (Alabaster, AL). Deuterium-depleted water was from Aldrich Chemical Co. (Milwaukee, WI). All the lipids were stored as lyophilized powders at –20 °C under nitrogen.

Sample Preparation. Multilamellar vesicles were prepared by dispersing in deuterium-depleted water dry and thin films of the DMPC-d_{54}–DSPC or DMPC–DSPC-d_{70} mixtures obtained by evaporation of $CHCl_3/CH_3OH$ (2:1 v/v) solutions containing the desired amounts of the lipid components. Complete hydration of the phospholipid mixtures was ensured during the process of lipid dispersal by thorough vortex mixing at 70 °C. The lipid dispersion was transferred to 5-mm diameter glass tubes and concentrated by centrifugation in a bench centrifuge.

NMR Spectroscopy. NMR spectra were recorded on a Nicolet NT-360B spectrometer running at 55 MHz for the ^{2}H nucleus. A fixed frequency high-power variable temperature probe, HP-50 from Cryomagnet Systems (Indianapolis, IN), with a 5-mm solenoid was used for the solid-state ^{2}H NMR experiments. The sample temperature was controlled by a Nicolet variable temperature accessory and was measured by inserting a thermocouple directly into the sample chamber. The quadrupole echo pulse sequence (Davis et al., 1976; Bloom et al., 1980) was used with a 90° pulse width of 2.4 μs. A relaxation delay of 300 ms and a spectral width of 1 MHz were used.

RESULTS

^{2}H NMR Spectra of Lipid Mixtures: Temperature Dependence. ^{2}H NMR spectra arising from equimolar DMPC-d_{54}–DSPC and DMPC–DSPC-d_{70} mixtures are shown in

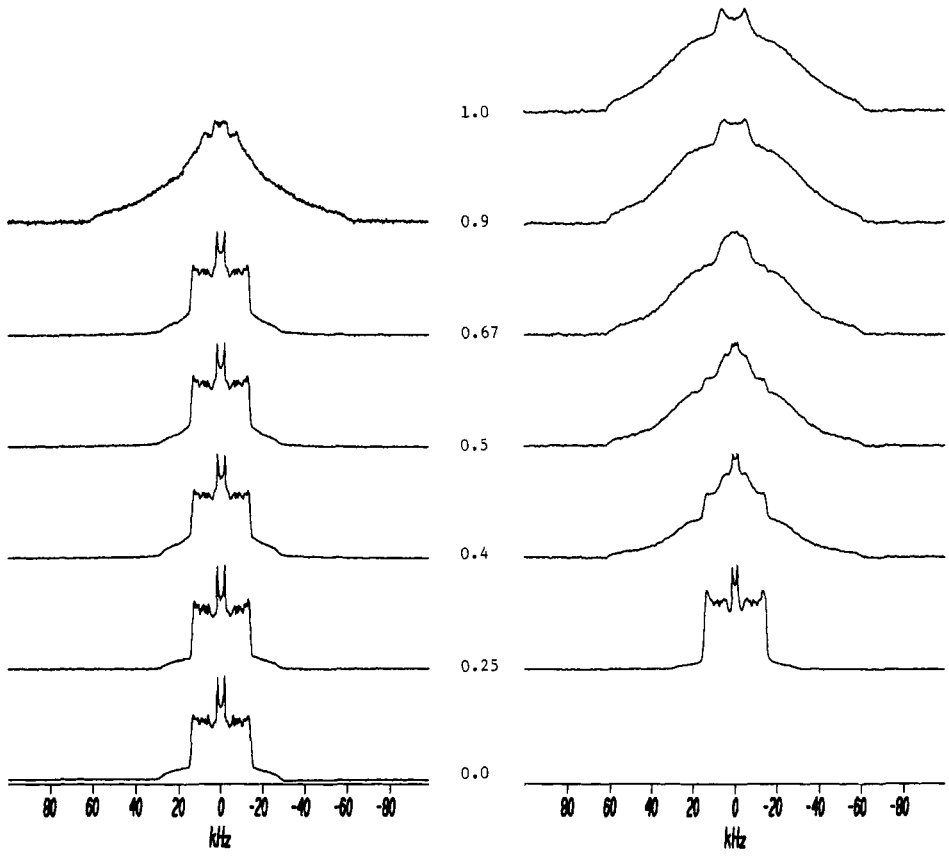

FIGURE 2: 55-MHz ^{2}H NMR spectra at different compositions of perdeuterated phospholipids in binary mixtures. The left panel shows the NMR spectra at 36 °C of mixtures of perdeuterated dimyristoylphosphatidylcholine, DMPC-d_{54}, and distearoylphosphatidylcholine, DSPC. The right panel shows the corresponding spectra at the same temperature obtained from mixtures of dimyristoylphosphatidylcholine, DMPC, with perdeuterated distearoylphosphatidylcholine, DSPC-d_{70}. The numbers between the two panels are the mole fractions of DSPC, X_{DSPC}, (for the left panel) or of DSPC-d_{70}, $X_{DSPC\text{-}d_{70}}$ (for the right panel).

Figure 1. At 22 °C, the spectrum from either mixture is a powder pattern characteristic of a gel phase [see Davis (1983) for a review]. At 60 °C, the spectra from both the mixtures correspond to a fluid-phase bilayer and are axially symmetric with resolved quadrupole splittings arising from individual methylene segments in the acyl chains. At intermediate temperatures, the spectra are superpositions of the gel- and fluid-phase spectra. As seen in this figure, the DMPC-d_{54}–DSPC system (Figure 1, left panel) contains, at any given temperature in the gel–fluid coexistence region, more fluid spectral component than the DMPC–DSPC-d_{70} system (Figure 1, right panel).

^{2}H NMR Spectra of Lipid Mixtures: Concentration Dependence. Figure 2 shows the dependence on lipid composition at a fixed temperature (36 °C) of the ^{2}H NMR spectra from DMPC-d_{54}–DSPC and DMPC–DSPC-d_{70} mixtures. Increasing the proportion of DSPC in DMPC-d_{54}–DSPC mixtures results in the conversion of a fluid-phase spectrum arising from the DMPC-d_{54} component at low DSPC content to a gel-phase spectrum at high DSPC content, through a two-spectral-component region at intermediate compositions (Figure 2, left panel). A similar change was observed when the spectra of the DSPC-d_{70} component in DMPC–DSPC-d_{70} mixtures were recorded with increasing DSPC-d_{70} content in the system (Figure 2, right panel). At any given lipid composition, the spectra from mixtures containing DMPC-d_{54} comprise a higher proportion of the fluid spectral component than those from mixtures containing DSPC-d_{70}.

Quantitation of the Gel and Fluid Phases. The appearance of resolved gel and fluid spectral components in the ^{2}H NMR spectra for both the mixtures means that the exchange between the two phases of both the component molecules is slow on the ^{2}H NMR time scale. The two-component spectra at different temperatures and lipid compositions such as those shown in Figures 1 and 2 can be analyzed by either of two methods to define the boundaries of the two-phase coexistence region. The first method involves the use of the spectral first moments, M_1. The first moment is defined as

$$M_1 = \frac{\int_0^\infty \omega f(\omega)\, d\omega}{\int_0^\infty f(\omega)\, d\omega} \qquad (1)$$

where $f(\omega)$ is the intensity at frequency ω [see Davis (1979)].

Upon heating, M_1 is known to drop suddenly at the gel–fluid phase transition in one-component lipid bilayers (Davis, 1979). Figure 3 panels and A and B show the dependence of M_1 on temperature for equimolar mixtures of DMPC-d_{54}–DSPC and DMPC–DSPC-d_{70}, respectively. The solidus boundary line was obtained from an analysis of the moment as the temperatures at which M_1 drops suddenly. The conversion of the entire system to the fluid phase at the fluidus boundary line was taken to occur at the temperature above which M_1 stabilizes at the fluid-phase value.

The second method involves the use of ^{2}H NMR difference spectroscopy (Vist & Davis, 1990; Morrow et al., 1991). In this method, a pair of normalized two-component spectra that contain different proportions of the gel and fluid components at a given temperature is chosen. Subtracting a fraction, K, of a spectrum from the DMPC-d_{54}–DSPC system at a given temperature with less gel component from the one with more

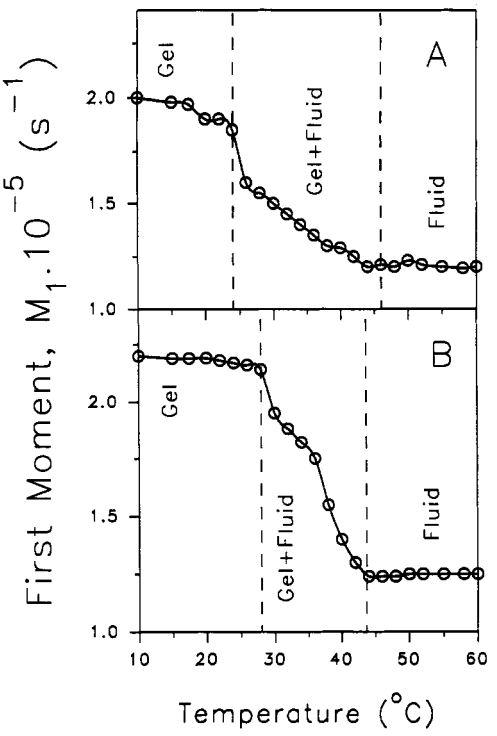

FIGURE 3: Dependence of the spectral first moments, M_1, on temperature for the two complementary equimolar mixtures formed from (A) DMPC-d_{54} and DSPC and from (B) DMPC and DSPC-d_{70}. The vertical dashed lines indicate the solidus and the fluidus phase boundaries. The solid lines were drawn to guide the eye.

gel component from the same system at the same temperature gives a difference spectrum that corresponds to the gel phase in the two spectra (results not shown). If X^a_{DSPC} and X^b_{DSPC} are the mole fractions of DSPC in the samples that give the two spectra that differ in the proportion of gel phase, then the mole fraction of DSPC, X_s, corresponding to the difference spectrum is given by (Vist & Davis, 1990)

$$X_s = \frac{(1-K)X^a_{DSPC}X^b_{DSPC}}{X^b_{DSPC} - KX^a_{DSPC}} \quad (2)$$

The opposite subtraction gives a fraction K' of the less-fluid spectrum to be subtracted from the more-fluid spectrum to give an endpoint that corresponds to the fluid phase in both the spectra. The mole fraction of DSPC corresponding to the endpoint spectrum, X_F, is given by

$$X_F = \frac{(1-K')X^a_{DSPC}X^b_{DSPC}}{X^a_{DSPC} - K'X^b_{DSPC}} \quad (3)$$

X_s and X_F are the mole fractions of DSPC in the gel-component spectrum and the fluid-component spectrum and hence are the points of intersection of the tie line with the solidus and the fluidus on the phase diagram, respectively (Vist & Davis, 1990). Using a similar set of relations for the DMPC–DSPC-d_{70} system, the mole fraction of DSPC-d_{70} at the solidus, X_s', and at the fluidus, X_F', were determined.

Temperature-Composition Phase Diagrams. The temperature–composition phase diagrams obtained for the DMPC-d_{54}–DSPC and the DMPC–DSPC-d_{70} systems are shown in Figure 4A,B. The solidus and the fluidus boundary lines were obtained using the methods of spectral first moments and of difference spectroscopy described above. The difference spectroscopic method was found to yield reliable endpoints only up to the temperature range of 40–45 °C. At higher temperatures, presumably due to increased exchange rates of the component molecules between the two phases, the endpoint spectra have shapes that depend on the lipid composition of the two-component spectra (Morrow et al., 1991). As seen in Figure 4, the phase diagrams for the two complementarily deuterated systems are similar in shape but are quantitatively different. The differences between the two systems can be understood in greater detail when the relative proportions of the two phases and of the two component molecules in each phase are compared. To do so, we rely on the fact that the fraction of the total lipid in the gel and fluid phases, and the fraction of either component in the two phases can be determined using the lever rule [see, e.g., Gordon (1968) and Cevc and Marsh (1987)]. For the DMPC-d_{54}–DSPC mixture (Figure 4A), the fraction of total lipid in the fluid phase, F, is given by

$$F = \frac{X_S - X_{DSPC}}{X_S - X_F} \quad (4)$$

where X_{DSPC} is the mole fraction of DSPC in the mixture. X_S and X_F are determined by the method of spectral subtraction applied to the DMPC-d_{54}–DSPC system using eqs 2 and 3 and by monitoring the spectral first moments as a function of temperature. The fraction of DSPC in the fluid phase, f_{DSPC}, is given by

$$f_{DSPC} = \frac{FX_F}{X_{DSPC}} \quad (5)$$

The fraction of DMPC-d_{54} in the fluid phase, $f_{DMPC\text{-}d_{54}}$, is determined by replacing the denominator in eq 5 by $X_{DMPC\text{-}d_{54}}$. For the DMPC–DSPC-d_{70} mixture (Figure 4B), the fraction of total lipid in the fluid phase, F', and the fraction of DSPC-d_{70} in the fluid phase, $f'_{DSPC\text{-}d_{70}}$, are given by

$$F' = \frac{X'_S - X_{DSPC\text{-}d_{70}}}{X'_S - X'_F} \quad (6)$$

$$f'_{DSPC\text{-}d_{70}} = \frac{F'X'_F}{X_{DSPC\text{-}d_{70}}} \quad (7)$$

where $X_{DSPC\text{-}d_{70}}$ is the mole fraction of DSPC-d_{70} in the mixture. X'_S and X'_F are the mole fractions of DSPC-d_{70} at the solidus and the fluidus, which are determined by spectral subtraction and moments analyses of the DMPC–DSPC-d_{70} system.

For carrying out a comparative analysis of the fluid fraction, and the composition of the fluid phase for the DMPC-d_{54}–DSPC and DMPC–DSPC-d_{70} systems, normalized fractional differences in these quantities will be defined. The dependence on X_{DSPC} at different temperatures of the fractional difference, ΔF, of the fraction of the total lipid in the fluid phase of the DMPC-d_{54}–DSPC system and that of the total lipid in the DMPC–DSPC-d_{70} system, $\Delta F = (F - F')/F$, are shown in Figure 5A. The bottom panel of this figure (Figure 5B) shows as a function of X_{DSPC} the fractional difference in the relative composition of the fluid phases in the two systems. This difference is defined as

$$\Delta(f_{DSPC}/f_{DMPC}) = [(f_{DSPC}/f_{DMPC\text{-}d_{54}}) - (f'_{DSPC\text{-}d_{70}}/f'_{DMPC})]/(f_{DSPC}/f_{DMPC\text{-}d_{54}})$$

It is seen from Figure 5A that the DMPC-d_{54}–DSPC system contains a larger fraction of total lipids in the fluid phase. However, the normalized ratio of DSPC-d_{70} (or DSPC) to DMPC (or DMPC-d_{54}) in the fluid phase, $\Delta(f_{DSPC}/f_{DMPC})$,

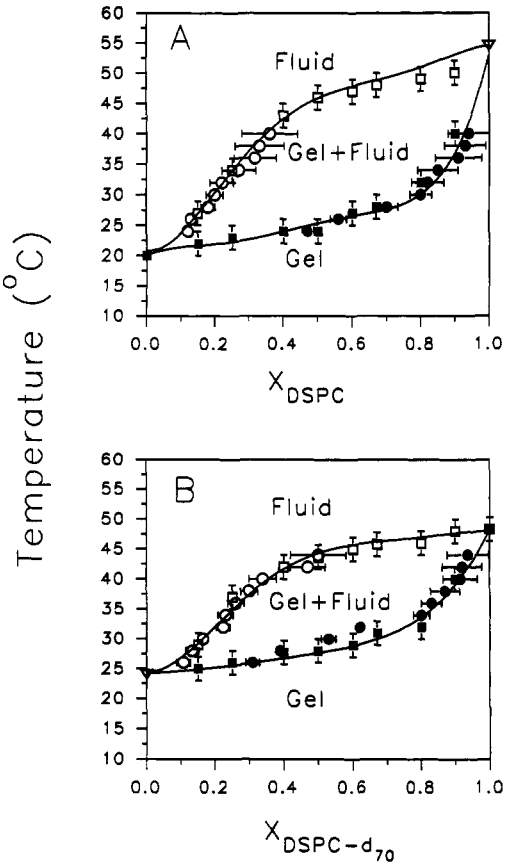

FIGURE 4: Temperature–composition phase diagrams for (A) the DMPC-d_{54}–DSPC system and for (B) the DMPC–DSPC-d_{70} system. The phase boundaries were obtained from spectral subtractions (○, ●) and from an analysis of the temperature dependence of the first moments, M_1 (□, ■), such as those shown in Figure 3. The filled and open symbols in both panels A and B correspond to the solidus and the fluidus, respectively. The vertical error bars are estimated errors associated with the determination of the inflexion points from the first moment data. The horizontal error bars were calculated as described by Morrow et al. (1991). The solid lines form the loci through the points.

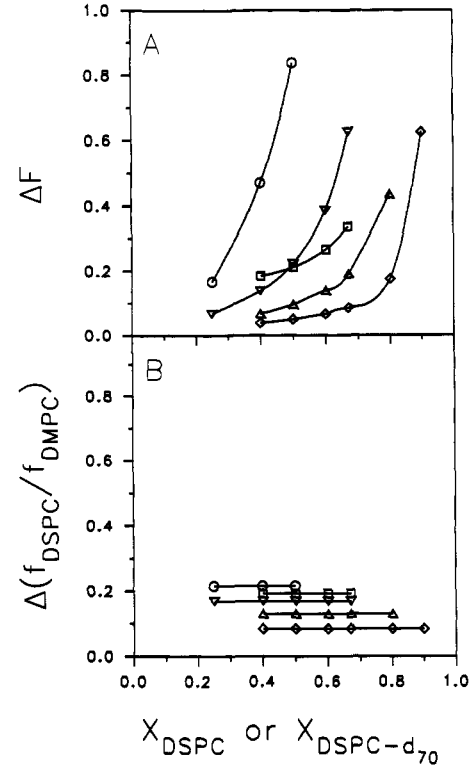

FIGURE 5: Effect of perdeuteration on phase and lipid composition of the fluid phase. (A) Fractional change in the fluid fraction, $\Delta F = (F - F')/F$, between the DMPC-d_{54}–DSPC (F obtained from Figure 4A) and the DMPC–DSPC-d_{70} (F' obtained from Figure 4B) systems. (B) Fractional change, $\Delta(f_{DSPC}/f_{DMPC}) = [(f_{DSPC}/f_{DMPC\text{-}d_{54}}) - (f'_{DSPC\text{-}d_{70}}/f'_{DMPC})]/(f_{DSPC}/f_{DMPC\text{-}d_{54}})$, in the ratio of DSPC to DMPC-d_{54} in the fluid phase (f_{DSPC} and $f_{DMPC\text{-}d_{54}}$ determined from Figure 4A) and the ratio of DSPC-d_{70} to DMPC in the fluid phase (f'_{DMPC} and $f'_{DSPC\text{-}d_{70}}$ determined from Figure 4B). Temperatures were 26 (○), 28 (▽), 30 (□), 34 (△), and 36 °C (◇). At each temperature, the data points were joined by a line to guide the eye.

undergoes relatively small changes with X_{DSPC} (Figure 5B) compared to the fluid fraction.

Acyl Chain Lengths of DMPC-d_{54} and DSPC-d_{70} in Binary Mixtures. The fluid-phase spectra of DMPC-d_{54} in DMPC-d_{54}–DSPC mixtures and of DSPC-d_{70} in DMPC–DSPC-d_{70} mixtures give resolved quadrupole splittings, $\Delta\nu_Q(i)$, corresponding to the various methylene and methyl segments, i, in the acyl chains. The $\Delta\nu_Q(i)$ values are linearly related to the segmental order parameters, $S_{CD}(i)$, as

$$\Delta\nu_Q(i) = \frac{3}{4}\frac{e^2qQ}{h}S_{CD}(i) \qquad (8)$$

where e^2qQ/h is the deuteron quadrupole splitting constant (Seelig & Seelig, 1980; Davis, 1983). The $S_{CD}(i)$ values may be used to calculate the effective average acyl chain length, $\langle L\rangle$, from position $(m-1)$ to n, the terminal methyl carbon, using the expression

$$\langle L\rangle = l\left[\left[\frac{n-m+1}{2}\right] - \sum_{i=m}^{n-1} S_{CD}(i) - 3S_{CD}(n)\right] \qquad (9)$$

where l is the projected length of an individual chain segment in the all-trans reference state (Seelig & Seelig, 1974; Salmon et al., 1987). $S_{CD}(i)$ values are presumed to be negative (Seelig, 1977). For DMPC-d_{54} (n = 14) and DSPC-d_{70} (n = 18), $\Delta\nu_Q(i)$ for the range i = 3–n were measured from the NMR spectra. The dependence on temperature at various lipid compositions of the acyl chain length from position 2 to the terminal methyl group at position 14 for DMPC-d_{54}, $\langle L\rangle^{2\text{-}14}$, and the length from position 2 to the terminal methyl group at position 18 for DSPC-d_{70}, $\langle L\rangle^{2\text{-}18}$, calculated using eqs 8 and 9 are shown in Figure 6, panels A and B, respectively. The $\langle L\rangle^{2\text{-}18}$ values are seen in this figure to decrease more steeply with temperature than the $\langle L\rangle^{2\text{-}14}$ values. The results are plotted at different temperatures as a function of the lipid composition in Figure 7. $\langle L\rangle^{2\text{-}18}$ (Figure 7B) increases more rapidly with increasing DSPC-d_{70} content than does $\langle L\rangle^{2\text{-}14}$ (Figure 7A).

Thickness of the Bilayer in the Fluid Phase. The average acyl chain lengths of the two components of the binary system described in Figures 6 and 7 can be used to obtain the thickness of the fluid phase bilayer, d, if the composition of the fluid phase is known. When the system is all fluid, the composition of the fluid phase is given by the initial composition of the lipid mixture. To treat the fluid phase when the system is all fluid and the fluid phase when the system is in the gel–fluid coexistence region by a single set of equations, a new variable x, the mole fraction of the longer-chain lipid (DSPC or DSPC-d_{70}) in the fluid phase, will be introduced. When the mixture under consideration is in the fluid phase, $x = X_{DSPC}$ for Figure 4A and $x = X_{DSPC\text{-}d_{70}}$ for Figure 4B. In the gel–fluid coexistence region, the fluid-phase composition can be determined from the phase diagram using the lever rule. In this case, $x = f_{DSPC}/(f_{DMPC\text{-}d_{54}} + f_{DSPC})$ for Figure 4A and $x = f'_{DSPC\text{-}d_{70}}/(f'_{DMPC} + f'_{DSPC\text{-}d_{70}})$ for Figure 4B. As mentioned

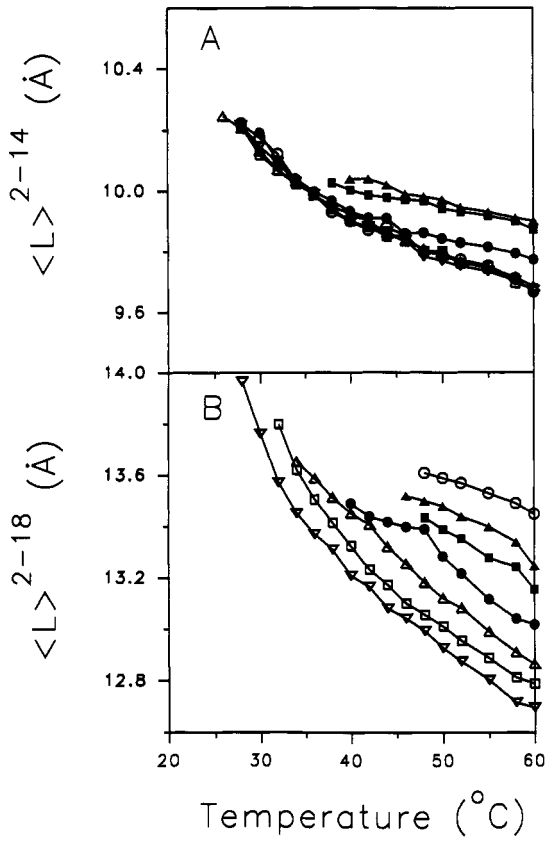

FIGURE 6: Dependence on temperature of the average acyl chain lengths of the *sn*-1 and *sn*-2 chains, spanning from the acyl chain carbon atom next to the carbonyl group to the terminal methyl group, n (n = 14 for DMPC-d_{54} and n = 18 for DSPC-d_{70}). (A) Acyl chain lengths, $\langle L \rangle^{2-14}$, obtained from the spectra of DMPC-d_{54}–DSPC mixtures. The mole fractions of DSPC, X_{DSPC}, were 0.00 (○), 0.15 (▽), 0.25 (□), 0.40 (△), 0.60 (●), 0.80 (■), and 0.90 (▲). (B) Acyl chain lengths, $\langle L \rangle^{2-18}$, obtained from DMPC–DSPC-d_{70} mixtures. The mole fractions of DSPC-d_{70}, $X_{DSPC\text{-}d_{70}}$, were 1.00 (○), 0.15 (▽), 0.25 (□), 0.40 (△), 0.60 (●), 0.80 (■), and 0.90 (▲). At each composition, the temperature dependence can be followed by following the lines drawn through the data points.

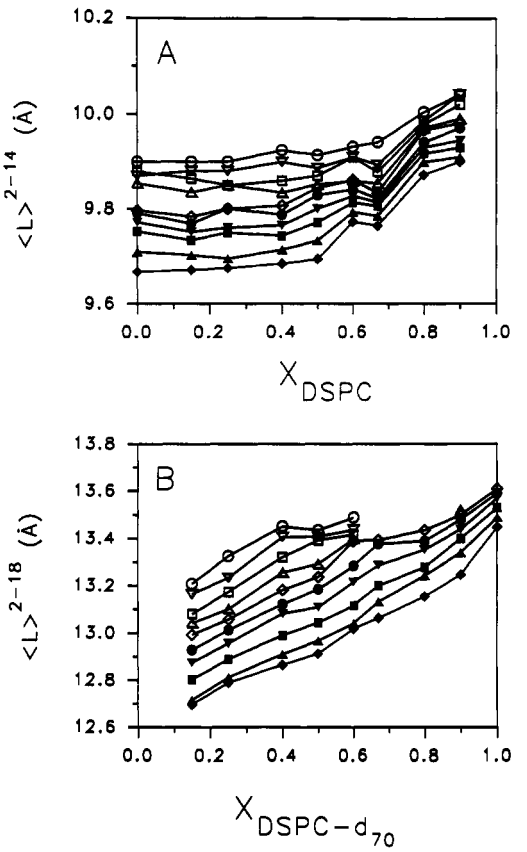

FIGURE 7: Dependence on the mole fraction, X_{DSPC}, of DSPC (A) or of $X_{DSPC\text{-}d_{70}}$ (B) of the average acyl chain lengths at different temperatures: 40 (○), 42 (▽), 44 (□), 46 (△), 48 (◇), 50 (●), 52 (▼), 55 (■), 58 (▲), and 60 °C (◆). (A) $\langle L \rangle^{2-14}$ for DMPC-d_{54}–DSPC mixtures. (B) $\langle L \rangle^{2-18}$ for DMPC–DSPC-d_{70} mixtures. Note that the y axis spans a range of 0.6 Å for panel A and 1.2 Å for panel B. Values for the chain length at different temperatures were connected by lines for visual guidance.

above, the phase diagram and hence the extent of gel–fluid immiscibility depends on which of the two component phospholipids is perdeuterated. Therefore, in the following, the fluid-phase bilayer thickness will be calculated using both of the phase diagrams.

It is seen in Figures 6 and 7 that acyl chain lengths of the DMPC-d_{54} molecule are always smaller than those of DSPC-d_{70}. This mismatch in chain lengths must result in time-averaged packing configurations for the lipid molecules in a fluid-phase bilayer that depend on the relative numbers of the short and long molecules. Figure 8 schematically illustrates the way in which the ends of the hydrocarbon chains of the two molecules can be aligned for 2:1, 1:1, and 1:2 DMPC/DSPC mixtures, in order to give flat bilayer surfaces, i.e., with the polar headgroups lying in a single plane. Over the entire composition range, this mode of packing results in a partially interdigitated structure for the bilayer. The schematic model presumes that there is no phase separation in the fluid phase of this binary system. Although the existence of two immiscible fluid phases has been established for cholesterol-containing binary mixtures (Sankaram & Thompson, 1990, 1991), there is no firm evidence for the existence of this phenomenon for the DMPC/DSPC system. The method of calculating the bilayer thickness from acyl chain lengths for binary mixtures comprised of flexible (phospholipid) and rigid (cholesterol) molecules in different proportions has been

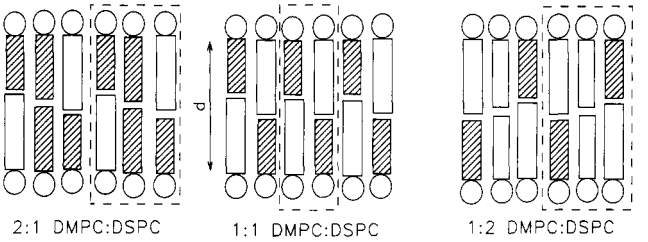

FIGURE 8: Schematic representation of a possible characteristic time-averaged arrangement of the DMPC and DSPC molecules. The circles and the boxes represent the headgroup and the hydrocarbon region of the phospholipid molecules. The hydrocarbon region of DMPC is shown as the hatched box. DMPC is the shorter molecule, and DSPC is shown as the longer molecule, based on the chain lengths determined for the two molecules in perdeuterated form in binary mixtures (Figures 6 and 7). The headgroups are aligned allowing for a mismatch at the hydrocarbon termini, for 2:1, 1:1, and 1:2 mol/mol DMPC/DSPC mixtures. For calculating the bilayer thickness, d, each mixture is treated as being composed of a characteristic repeating unit which for each of the three illustrations is enclosed in dashed boxes. See Sankaram and Thompson (1990) for a more detailed description of the method.

described in detail (Sankaram & Thompson, 1990, 1991). This method is now generalized for the case of arranging two flexible molecules in a bilayer. According to this method, the bilayer thickness, d, for a mixture composed of DMPC and DSPC in different proportions (when the DMPC is the major component in the system, i.e., when $x < 0.5$) can be considered to be an average of the thickness calculated for a DMPC–DMPC pair, d_0, and for a DMPC–DSPC pair, $d_{1:1}$. The

average thickness, d, is given by

$$d = (1 - 2x)d_0 + 2xd_{1:1} \quad (10)$$

where $d_0 = 2(\langle L\rangle_x^{2-14} - x\langle L\rangle_{x=0.5}^{2-14})/(1 - x)$ and $d_{1:1} = \langle L\rangle_{x=0.5}^{2-14} + \langle L\rangle_x^{2-18}$ [see eq 8 in Sankaram and Thompson (1990)]. $\langle L\rangle_x^{2-14}$, $\langle L\rangle_x^{2-18}$, and $\langle L\rangle_{x=0.5}^{2-14}$ are the acyl chain lengths of DMPC-d_{54} at a composition x, of DSPC-d_{70} at a composition x, and of DMPC-d_{54} for an equimolar mixture, respectively.

When DSPC is the major component in the system, the average is performed over the thickness contributions obtained for a DSPC–DSPC pair, d_0, and for a DMPC–DSPC pair, $d'_{1:1}$. In this case, the average thickness, d, is given by

$$d = (1 - 2x)d_0' + 2xd'_{1:1} \quad (11)$$

where $d_0' = 2(\langle L\rangle_x^{2-18} - (1-x)\langle L\rangle_{x=0.5}^{2-18})/x$, $d'_{1:1} = \langle L\rangle_x^{2-14} + \langle L\rangle_{x=0.5}^{2-18}$ and $\langle L\rangle_{x=0.5}^{2-18}$ is the acyl chain length of DSPC-d_{70} for an equimolar mixture. Equations 10 and 11 can be reduced to the following general expressions that relate the bilayer thickness, d, to composition and to the individual acyl chain lengths. If $x < 0.5$,

$$d = \frac{2}{1-x}[(1 - 2x)\langle L\rangle_x^{2-14} + x^2\langle L\rangle_{x=0.5}^{2-14} + x(1 - x)\langle L\rangle_x^{2-18}] \quad (12)$$

If $x > 0.5$

$$d = \frac{2}{x}[(2x - 1)\langle L\rangle_x^{2-18} + (1 - x)^2\langle L\rangle_{x=0.5}^{2-18} + x(1 - x)\langle L\rangle_x^{2-14}] \quad (13)$$

For an equimolar mixture ($x = 0.5$), both eqs 12 and 13 for this model reduce to

$$d = \langle L\rangle_x^{2-14} + \langle L\rangle_x^{2-18} \quad (14)$$

Also, when both the molecules have the same acyl chain length, i.e., when $\langle L\rangle = \langle L\rangle_x^{2-14} = \langle L\rangle_x^{2-18}$, eqs 12 and 13 reduce to $d = 2\langle L\rangle$. In this case, the problem is reduced to that for a one-component system, and, accordingly, eqs 12 and 13 give the thickness as twice the acyl chain length. Thus, the above set of eqs 12 and 13 have the appropriate limiting behavior.

The thickness of the fluid-phase bilayer, d, for the DMPC-d_{54}–DSPC system calculated using eqs 12 and 13 is shown in Figure 9A. For comparison, d values obtained by using the phase diagram for the DMPC–DSPC-d_{70} system are given in Figure 9B. The data shown in Figure 9A are plotted in Figure 10 as a function of the mole fraction of DSPC, x, at four different temperatures: one corresponding to the all-fluid, one-phase region of both the phase diagrams and three to the fluid phases in the gel–fluid coexistence region. The values for d obtained for the DMPC-d_{54}–DSPC system (Figure 9A) are nearly identical to those obtained for DMPC–DSPC-d_{70} (Figure 9B).

DISCUSSION

In this work, the temperature-composition phase diagrams were constructed from an analysis of the spectral moments and difference spectroscopy. The mean chain lengths of the myristoyl and stearoyl chains of the component phospholipid molecules in the binary mixture were used in conjunction with the phase diagrams to obtain the thickness of the bilayer in the fluid phase.

Effect of Perdeuteration on Phase Behavior. The temperature–composition phase diagram of the DMPC–DSPC-d_{70} system shown in Figure 4B is in good agreement with that reported recently using ^{2}H NMR difference spectroscopy (Morrow et al., 1991). Interestingly, the phase diagram of

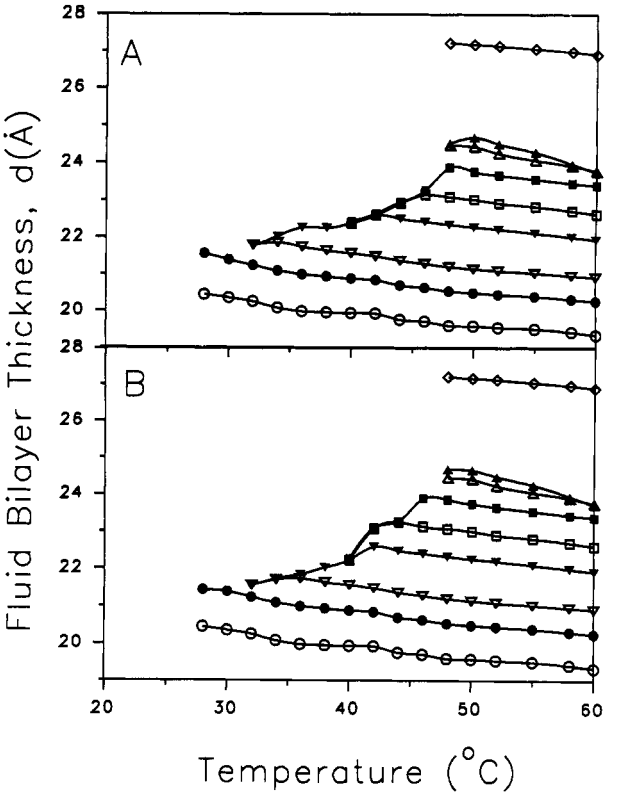

FIGURE 9: Thickness, d, of the fluid-phase mixed bilayer as a function of temperature at different mole fractions of DSPC, X_{DSPC}. (A) DMPC-d_{54}–DSPC mixtures. d was calculated using eqs 12 and 13, and the chemical composition of the fluid phase was determined from Figure 4A. X_{DSPC} were 0.00 (○), 0.15 (●), 0.25 (▽), 0.40 (▼), 0.50 (□), 0.60 (■), 0.67 (△), 0.80 (▲), and 1.00 (◇). (B) DMPC–DSPC-d_{70} mixtures. d was calculated using eqs 12 and 13 and Figure 4B. The various symbols correspond to the same numerical values given for panel A. Lines were drawn through the data points for clarity.

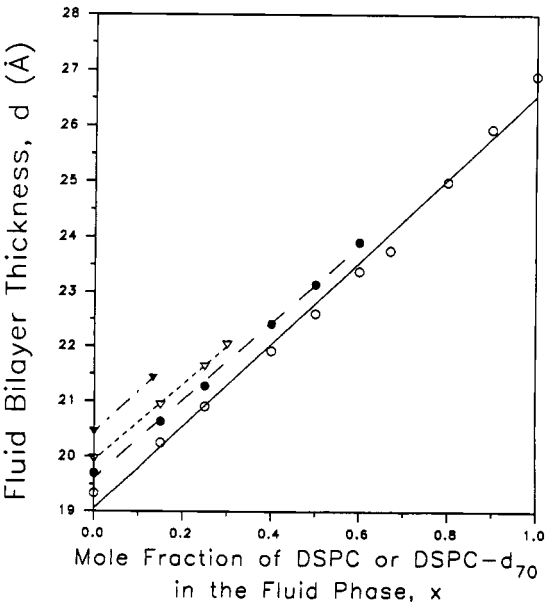

FIGURE 10: Thickness, d (Å), of the fluid-phase mixed bilayer as a function of the mole fraction, x, of DSPC. Temperatures were 60 (○, —), 46 (●, – – –), 38 (△, - - -), and 28 °C (▲, —·—). Note that at 60 °C the system is all fluid. At the other three temperatures, both the gel and the fluid phases coexist. The lines through the data points are obtained by linear regression.

the DMPC-d_{54}–DSPC system (Figure 4A) differs markedly from this phase diagram. In the DMPC-d_{54}–DSPC system, the gel–fluid coexistence region extends over a larger temperature and composition interval, i.e., covers a larger area on the temperature–composition phase diagram than in the DMPC–DSPC-d_{70} mixtures. This is not altogether surprising since the difference in the melting temperatures of the pure components is 35 °C when DMPC is perdeuterated and 28 °C when DSPC is perdeuterated. While the extent of gel–fluid coexistence is thus dependent on which chemical constituent is perdeuterated, there is no evidence in the ^{2}H NMR spectra of any gel–gel coexistence in either mixture in the temperature interval 10–24 °C (see Figure 4A,B). Contrary to this observation, preliminary results using spin-label electron spin resonance spectroscopy (M. B. Sankaram and T. E. Thompson, unpublished observations) suggest the existence of gel–gel coexistence for all the three systems, namely, DMPC–DSPC, DMPC-d_{54}–DSPC, and DMPC–DSPC-d_{70}. It is likely that the spectral resolution afforded by ^{2}H NMR does not adequately resolve the different gel phases. It should be noted that whether the phase diagram of the DMPC–DSPC system is monotectic (absence of gel–gel coexistence) or peritectic (presence of gel–gel coexistence) is a much debated topic in the literature (Mabrey & Sturtevant, 1976; Knoll et al., 1981; Morrow et al., 1991). A different approach directly looking for gel–gel immiscibility is required to clarify this issue.

Perdeuteration is known to decrease the melting temperatures of one-component lipid bilayers by about 4 °C. This observation has prompted the practice of simply offsetting the temperature–composition phase diagrams obtained on perdeuterated systems by an equivalent amount to higher temperatures for the purpose of either comparing the data obtained on nondeuterated systems or of determining a phase diagram for the nondeuterated system (Knoll et al., 1981; Ipsen et al., 1987). The results presented in this paper clearly show that this procedure is only an approximation which ignores the differences in the fluid fraction and the composition of the gel and fluid phases involved (see Figures 4 and 5).

The differences in the phase behavior for the two binary mixture investigated here may be compared at a more detailed level if the fraction of total lipids in either phase and the relative proportions of the two lipids *within* a given phase are compared for the DMPC-d_{54}–DSPC and the DMPC–DSPC-d_{70} systems (in the gel–fluid coexistence region). These parameters are easily obtained from the phase diagrams using the lever rule (cf. Results). First, the fraction of the total lipids at any given temperature in the fluid phase, F, is larger by up to 90% for the DMPC-d_{54}–DSPC system than for the DMPC–DSPC-d_{70} system (Figure 5A). When F obtained from the phase diagram of DMPC–DSPC (Mabrey & Sturtevant, 1976; Knoll et al., 1981) was compared with that obtained from Figure 4A,B, it was found that F decreases in the order DMPC-d_{54}–DSPC > DMPC–DSPC > DMPC–DSPC-d_{70} (data not shown). Second, the maximal variation in the composition of the fluid phase in the gel–fluid coexistence region for the DMPC–DSPC (not shown), DMPC-d_{54}–DSPC (Figure 5C), and DMPC–DSPC-d_{70} (Figure 5C) systems is about 20%, while F changes up to 90%. In other words, perdeuteration of lipids has a strong effect on the extent of formation of the fluid phase while minimizing the associated alterations in the lipid composition of that phase. It is interesting to note that both F and the difference in the main transition temperatures of the individual one-component systems decrease in the order DMPC-d_{54}–DSPC (~35 °C) > DMPC–DSPC (~31 °C) > DMPC–DSPC-d_{70} (~28 °C).

Acyl Chain Lengths in Binary Lipid Mixtures. The effective acyl chain lengths, $\langle L \rangle$, were determined from the resolved quadrupole splittings, $\Delta\nu_Q(i)$, in the fluid-phase ^{2}H NMR spectra, assuming a linear relation relationship between $\Delta\nu_Q(i)$ and $\langle L \rangle$ (cf. eqs 8 and 9). Equivalently, the first moments, M_1, of the axially symmetric powder patterns may be related linearly in much the same way to $\langle L \rangle$ [see, e.g., Ipsen et al. (1990)]. In this work, the former method was employed since, in practice, it is normally possible to obtain accurate quadrupole splitting measurements while the M_1 values depend strongly on the signal-to-noise ratio of the spectra (Davis, 1979). In studies on two-component, two-phase systems such as those reported in this paper, the fluid *difference* spectra usually contain considerable baseline noise preventing the use of the first moments for the determination of effective acyl chain lengths.

Dependence on Composition and Temperature of the Average Acyl Lengths. The dependence of the effective acyl chain length of DMPC-d_{54} on temperature and composition is different from that of DSPC-d_{70}. The length of the DMPC-d_{54} molecule in the entire temperature and composition range undergoes relatively small changes, by a maximum of 0.55 Å (see Figure 7A). In comparison, the average length of the DSPC-d_{70} molecule decreases with increasing temperature (and with decreasing mole fraction of DSPC-d_{70} in the mixtures) approximately twice more steeply, up to 1.3 Å (see Figure 7B). Thus, the DSPC-d_{70} molecules are more *deformable* in the lipid mixtures than are the DMPC-d_{54} molecules. To quantitatively compare the dependence of the effective acyl chain lengths on temperature and composition, one may define a coefficient of thermal expansion and a coefficient of compositional expansion for $\langle L \rangle$. The coefficient of thermal expansion (at a given system composition and constant pressure) for DMPC-d_{54}, α_T, is defined as $(1/\langle L \rangle^{2-14})\, \partial\langle L \rangle^{2-14}/\partial T$. The corresponding coefficient for DSPC-d_{70} is defined as $\alpha_T' = (1/\langle L \rangle^{2-18})\, \partial\langle L \rangle^{2-18}/\partial T$. The coefficients of compositional expansion are defined as $\alpha_x = (1/\langle L \rangle^{2-14})\, \partial\langle L \rangle^{2-14}/\partial x$ and $\alpha_x' = (1/\langle L \rangle^{2-18})\, \partial\langle L \rangle^{2-18}/\partial x$, where x is the mole fraction of DSPC in DMPC-d_{54}–DSPC mixtures or the mole fraction of DSPC-d_{70} in DMPC–DSPC-d_{70} mixtures. α_T, α_T', α_x, and $\alpha x'$ can be estimated from the temperature and composition dependence of ^{2}H NMR quadrupole splittings using eq 7. The coefficients are given in Table I, for one-component bilayers containing either DMPC-d_{54} or DSPC-d_{70} alone and for the two-component systems. Since the lipid composition in one-component systems is invariant, α_x and α_x' do not exist for those systems.

In one-component lipid bilayers, the coefficient of thermal expansion of the acyl chain length was measured for deuterated dipalmitoyl phosphatidylcholine bilayers using ^{2}H NMR spectroscopy (Seelig & Seelig, 1974; Salmon et al., 1987). The reported value for the coefficient was –0.0025 K^{-1} when specifically deuterated phospholipids were employed (Seelig & Seelig, 1974) and was –0.0015 K^{-1} for the perdeuterated system (Salmon et al., 1987). A value of –0.0015 K^{-1} was reported for 1-perdeuterio-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine bilayers (Salmon et al., 1987). In the present study, the coefficient of thermal expansion of –0.00154 K^{-1} measured for DMPC-d_{54} bilayers is in agreement with the literature data. However, the coefficient of thermal expansion for DSPC-d_{70} bilayers (–0.00291 K^{-1}) is greater than that for DMPC-d_{54} bilayers (see Table I). In the binary mixtures, addition of DSPC reduces the magnitude of the coefficient of thermal expansion for DMPC-d_{54}, α_T, while

Table I: Coefficients of Thermal (α_T, α_T', β_T) and Compositional Expansion (α_x, α_x', β_x) for the Acyl Chain Length of DMPC-d_{54} (α_T, α_x) and of DSPC-d_{70} (α_T', α_x') and for the Bilayer Thickness of the Fluid Phase (β_T, β_x)[a]

one-component bilayer		two-component bilayer					
		DMPC-d_{54}		DSPC-d_{70}			
DMPC-d_{54} α_T (K^{-1})	DSPC-d_{70} α_T' (K^{-1})	α_T (K^{-1})	α_x	α_T' (K^{-1})	α_x'	β_T (K^{-1})	β_x
−0.00154	−0.00291	−0.00151 + 0.00037x	0.01985	−0.00093 − 0.00151x	0.0587	−0.00154 − 0.00137x	0.3682

[a] $\alpha_T = (1/\langle L\rangle^{2-14})(\partial\langle L\rangle^{2-14}/\partial T)$, $\alpha_T' = (1/\langle L\rangle^{2-18})(\partial\langle L\rangle^{2-18}/\partial T)$, $\alpha_x = (1/\langle L\rangle^{2-14})(\partial\langle L\rangle^{2-14}/\partial x)$, $\alpha_x' = (1/\langle L\rangle^{2-18})(\partial\langle L\rangle^{2-18}/\partial x)$, $\beta_T = (1/d)(\partial d/\partial T)$, $\beta_x = (1/d)(\partial d/\partial x)$. $\langle L\rangle^{2-14}$ and $\langle L\rangle^{2-18}$ are the effective acyl chain lengths from position 2 to the terminal methyl group for DMPC-d_{54} and DSPC-d_{70}, respectively. T is absolute temperature, and x is the mole fraction of DSPC or DSPC-d_{70} in the binary mixtures. For the two-component bilayers, α_T, α_T', and β_T are composition dependent, while the temperature dependence of α_x, α_x', and β_x was found to be statistically insignificant.

addition of DSPC-d_{70} increases the magnitude of the coefficient of thermal expansion for DSPC-d_{70}, α_T'. Similarly, the coefficient of compositional expansion for DMPC-d_{54}, α_x, is less than half the value for DSPC-d_{70}, α_x'. In the entire temperature and composition range when the binary mixtures are in the all-fluid or in the gel–fluid coexistence region, the largest value obtained for the DMPC-d_{54} molecule in the fluid phase was smaller than the smallest value obtained for the DSPC-d_{70} molecule in the fluid phase. While the thermal expansion coefficients exhibit a significant composition dependence, the coefficients of compositional expansion for the two molecules in binary mixtures, α_x and α_x', do not depend very significantly on temperature. Thus, in Table I, α_x and α_x' are shown as constants.

Average Thickness of the Fluid-Phase Mixed Bilayer. To calculate the time- and weighted-average bilayer thickness from the acyl chain lengths, the average relative bilayer location along the direction perpendicular to the plane of the bilayer of one molecule with respect to the other must be considered. There are two ways in which two molecules of different lengths can be arranged in a bilayer. In one, the mismatch in the acyl lengths of the DMPC-d_{54} and DSPC-d_{70} molecules may result in an uneven or undulating surface where the polar headgroups of the two different molecules are all not in a plane, but the terminal methyl groups are in register in the center of the bilayer. This undulation-matched arrangement must lead to part of the hydrophobic region in the longer molecule, in this case DSPC-d_{70}, extending into the water phase, which results in unfavorable water–hydrocarbon contacts. Alternatively, the polar headgroups may be lined up, but the terminal methyl groups of the two different types of molecules do not meet at the center of the bilayer. This configuration leads to a partial interdigitation of the acyl chains, which does not suffer from an exposure to water of part of the hydrophobic region of DSPC-d_{70}. Thus, this latter model is used in this work to calculate the bilayer thickness.

In one-component fluid lipid bilayers, the chain length and the thickness are essentially equivalent since for a bilayer the latter is twice the value of the molecular length. The molecular length is the sum of the acyl chain length from position 2 to the terminal methyl carbon, $\langle L\rangle^{2-14}$ or $\langle L\rangle^{2-18}$, and the distance between the headgroup atoms (phosphorus or nitrogen) to position 2 in the acyl chain. In two-component systems, the acyl chain lengths must be appropriately weighted to obtain the thickness of the lipid bilayer. The ^{2}H NMR method used here defines the chain length and hence the thickness precisely in terms of the carbon atoms in the acyl chain between which the effective projected thickness is measured. Electron density profile measurements and X-ray diffraction define the bilayer thickness in the hydrocarbon region. It is necessary to assume a value for the hydrophilic contribution to the bilayer thickness determined from X-ray diffraction spacings, to obtain the hydrophobic thickness. The electron density profiles give the transbilayer interphosphate distances which should be reduced by using a different value for the contribution from the headgroup region, to obtain the hydrophobic thickness. The agreement of bilayer thickness determined using ^{2}H NMR with that obtained by the two other methods is therefore not expected to be exact. However, the relative changes in the thickness in response to temperature and composition changes are expected to be in good accordance among the three different methods. For one-component lipid bilayers, the temperature dependence of the bilayer thickness was found in a number of ^{2}H NMR investigations to be in agreement with X-ray diffraction measurements (Seelig & Seelig, 1974; Salmon et al., 1987; Sankaram & Thompson, 1990). We are not aware of X-ray diffraction or electron density studies investigating the temperature and composition dependence of the bilayer thickness in two-component, two-phase systems.

Values for the time- and weighted-average bilayer thickness calculated using the two phase diagrams given in Figure 4A,B show very similar trends when the temperature or the composition of the bilayer is changed (compare panels A and B in Figure 9). Although the phase diagrams for the DMPC-d_{54}–DSPC and the DMPC–DSPC-d_{70} systems are significantly different, the relative insensitivity of the bilayer thickness to the use of either phase diagram is because the relative proportions of the component molecules in the fluid phase of the two systems do not change much (Figure 5C). Despite this result, the better method of determining the bilayer thickness is to obtain the acyl chain lengths of the two component molecules and the phase diagram from a single set of measurements. If specifically deuterated phospholipids are employed, the reduced extent of deuteration would not be expected to change the phase behavior significantly. In such experiments, it should be possible to obtain the chain lengths of both the components which can be used in conjunction with a single phase diagram to determine bilayer thickness.

Dependence on Composition and Temperature of the Average Thickness. As expected, at any given temperature the thickness of the fluid-phase bilayer increases with increasing mole fraction of the DSPC component in the system. Also, the thickness increases with decreasing temperature at any given composition. The data in Figure 10 show that this composition and temperature dependence of d holds not only when the system is all fluid but also for the fluid phase in the gel–fluid coexistence region. Similar results were obtained for two other binary mixed systems, namely, DMPC–dipalmitoylphosphatidylcholine and phospholipid–cholesterol mixtures (M. B. Sankaram and T. E. Thompson, unpublished observations). Although the dependence of d on x as given by eqs 12 and 13 is nonlinear, d actually varies almost linearly with x as seen in Figure 10. This is because the x dependence of the length of DMPC-d_{54}, $\langle L\rangle^{2-14}$, is much smaller than that of $\langle L\rangle^{2-18}$ (Figure 7). If $\langle L\rangle_x^{2-14}$ in eqs 12 and 13 is held

constant, the dependence of d on x becomes linear. The nonlinear increase of d with x is thus expected to be most pronounced in systems where both components respond significantly to temperature and compositional variations.

The conceptually different meaning for the bilayer thickness for one- and two-component bilayers may be further analyzed in terms of the coefficients of thermal and compositional expansion of the bilayer thickness, d. In one- and two-component systems, the coefficient of thermal expansion of bilayer thickness is defined as $\beta_T = (1/d)\,\partial d/\partial T$. In a one-component system, β_T is given by α_T, since d is twice the acyl chain length $\langle L \rangle$. β_T for a binary mixture can be obtained from eqs 12 and 13. The coefficient of compositional expansion does not exist for one-component systems, whereas it can be defined for binary mixtures as $\beta_x = (1/d)\,\partial d/\partial x$. Table I gives β_T and β_x values calculated for the binary mixed systems investigated in this work. β_T increases linearly with lipid composition while β_x does not depend significantly on temperature. The data shown in Figure 10 and the coefficients of thermal and compositional expansions given in Table I provide a means of quantitatively relating the effect on d of decreasing temperature with that of increasing x. The temperature decrease, ΔT, required to increase the bilayer thickness by an amount, Δd, is given by $\Delta T = (\beta_x/\beta_T)\Delta x$. From the present set of data, it can be inferred that introduction of a small amount of a longer acyl chain lipid into the fluid phase of a bilayer membrane (which may coexist with another phase) results in a thickness increase which corresponds to a large temperature decrease. For example, when the mole fraction of DSPC, x, is increased from 0 to 0.1, d increases by 3.7%. To effect the same increase in d, at 39 K increase in temperature is required. Thus, in biological membranes the bilayer thickness may be modulated under isothermal conditions by metabolically controlled lipid compositional changes.

Average Molecular Volume, Molecular Area, and Bilayer Thickness. The bilayer thickness, d, the average cross-sectional area per molecule, A, and the molecular volume, V, are interrelated parameters that characterize a bilayer (Ipsen et al., 1990). The relationship is given by $V = Ad$. Using the β_T and β_x values given in Table I for the thickness and the values reported in the literature for the thermal and composition dependence of the molecular volumes in the DMPC-d_{54}–DSPC system (Schmidt & Knoll, 1986), the corresponding coefficients for the molecular area, A, can be calculated. Using mass densitometry, molecular volumes in the fluid phase at 70 °C of 1120 Å^3 for DMPC-d_{54} and 1360 Å^3 for DSPC and a temperature-induced increase of 0.8 Å^3/°C for the average molecular volume in the fluid phase were reported (Schmidt & Knoll, 1986). The molecular volume, V, measured in this work is an average over the volumes of the DMPC-d_{54} and DSPC. These measurements yield a coefficient of thermal expansion of the molecular volume, $(1/V)\,\partial V/\partial T$, of 0.000714–0.0001261$x$ K^{-1} and a coefficient of compositional expansion, $(1/V)\,\partial V/\partial x$, of 0.21429. These data, along with the data shown in Table I [corrected for the approximately 260 Å^3 contribution from the headgroup volume given in Wilkinson and Nagle (1981)], yield a coefficient of thermal expansion of the average cross-sectional area per molecule, $(1/A)\,\partial A/\partial T$, of 0.6143–0.1219$x$ K^{-1}. The coefficient of compositional expansion of A, $(1/A)\,\partial A/\partial x$, is calculated to be –0.7580.

Models for Binary Mixed Bilayers. A recent theoretical study (Ipsen et al., 1988) has attempted to calculate the bilayer thickness for a number of binary mixtures including the DMPC–DSPC system. The thickness of the fluid phase, of the gel phase, and the thickness of the entire bilayer averaged over both the gel and the fluid phases were calculated in this study. In the present investigation, only the thickness of the fluid-phase bilayer is dealt with. The bilayer thickness of the fluid phase in the gel–fluid coexistence region was calculated in the theoretical study by assuming that the lengths of the two component molecules are locked-in. The study predicted that the thickness of the fluid phase in the gel–fluid coexistence region does not change with increasing DSPC content in the system. The experimental data shown in Figure 10 do not agree with this prediction.

The origin for the discrepancy between the theoretical prediction and the experiment is in the assumption that the acyl chain lengths of the two different component molecules such as DMPC and DSPC are locked in. The term "lock in" indicates that the transbilayer projected length of two molecules in a binary mixture is the same although in one-component bilayers formed from either molecule the lengths are different. Alternatively, lock in can lead to a difference in the lengths of the two molecules in binary mixtures, but the area per either molecule becomes equal to that of the other. The results presented in this paper clearly show that both the effective acyl chain length and the area per molecule are different for the two molecules in the binary mixtures. They also show that the length and area parameters for either molecule are different in one- and two-component systems. The lock in is a feature of the mattress model (Mouritsen & Bloom, 1984) for lipid–protein interactions, which is a comprehensive model that successfully explains many experimental results on lipid–protein interactions. In this model, it has been suggested that the lipid bilayer is capable of adjusting its thickness to match that of the hydrophobic length of an embedded membrane protein. Of the two species, only the lipid molecules are flexible enough to adjust their chain lengths. Any adjustment in the transbilayer projected length of the protein component usually must involve major conformational alterations. However, in lipid mixtures both of the component lipid molecules are flexible such that both can undergo length alterations to produce a homogeneous lipid bilayer of a particular phase type. Thus, in principle, lipids with a smaller number of carbons per chain can increase their effective length by increasing the proportion of trans conformers while those with a larger number of carbons per chain can decrease their length by decreasing the trans conformer population to reach an equilibrium value. The former process is energetically more expensive since it produces order while the latter disordering process will be energetically favorable. However, in the alternately perdeuterated DMPC–DSPC system, only the length of the DSPC molecule changes significantly while that of DMPC remains essentially unaltered. This may be a more cost-effective mechanism of regulating membrane thickness than varying the effective lengths of both the molecules, since little energy expenditure is required to stretch the disordered and shorter DMPC molecules.

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