Smoothed Acyl Chain Orientational Order Parameter Profiles in Dimyristoylphosphatidylcholine-Distearoylphosphatidylcholine Mixtures: A ^{2}H-NMR Study

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ABSTRACT The accommodation of chain-length mismatch in liquid crystal phase bilayers was examined by using deuterium nuclear magnetic resonance to obtain smoothed orientational order parameter profiles for acyl chains of both components in binary lipid mixture bilayers. Mixtures of dimyristoylphosphatidylcholine (DMPC) and distearoylphosphatidylcholine (DSPC) covering a range of compositions were prepared with either DSPC acyl chains or DMPC acyl chains perdeuterated. Orientational order parameters in the plateau regions of the smoothed profiles for both components were found to increase smoothly with increasing DSPC concentration. The orientational order parameters in the DSPC-smoothed profile were found to be slightly higher than corresponding values for DMPC over a wide range of bilayer composition. The shapes of the smoothed profiles for both components were found to be sensitive to bilayer composition. At low DSPC concentration, DSPC methylene deuterons near the bilayer center display a secondary plateau at low orientational order. At high DSPC concentration, the plateau of the DMPC-smoothed profile is stretched slightly. The concentration dependence of the smoothed profiles at low DSPC concentration appears to be consistent with a picture in which the last few segments of the DSPC chain cross the bilayer midplane, on average, but remain very disordered.

INTRODUCTION

Biological membranes generally contain lipids having a range of hydrocarbon chain lengths. Chain length heterogeneity can influence collective properties such as phase behavior and mean bilayer thickness. Within such a bilayer, the mean behavior of a particular component, examined using a technique such as ^{2}H-NMR, can reflect the average environment sampled as molecules diffuse through the bilayer. Such information can provide insights into the way in which lipid acyl chains of varying length are accommodated in a bilayer. One indication of the potential significance of chain-length mismatch accommodation is the observation that fatty acid chain length affects the membrane recognition properties of glycosphingolipids (Grant, 1987).

Lipid mixtures in which there is a significant chain-length mismatch between components have been studied extensively. Some of this work has been reviewed elsewhere (Morrow et al., 1993; Slater and Huang, 1988). There is some agreement that within a variety of ordered bilayer phases, chain-length mismatch is accommodated by varying degrees of interdigitation wherein the longer chain extends across the bilayer midplane (Keough and Davis, 1979; Bunow, 1979; Bunow and Levin, 1980; Hui et al., 1984; Levin et al, 1985; Boggs and Mason, 1986; Huang and Mason, 1986; Reed and Shipley, 1987; Boggs et al., 1989; Stinson and Boggs, 1989). The term interdigitation has been also applied to the behavior of some chain-mismatched systems in the fluid phase (Grant et al., 1987; Melhorn et al., 1988). The accommodation of chain-length mismatch in fluid phase bilayers, however, is more difficult to characterize because, in general, detailed information regarding the distribution of acyl chain conformations is not accessible from measurements of mean properties.

Recently, ^{2}H-NMR has been used to obtain smoothed orientational order parameter profiles for small concentrations of glycosphingolipids with perdeuterated fatty acids having varying degrees of chain-length mismatch with a fluid phospholipid host bilayer (Lu et al., 1993; Morrow et al., 1993). The order parameter profile for a galactosyl ceramide (GalCer) having a perdeuterated 24-carbon acyl chain in bilayers of the 18-carbon chain phospholipid 1-stearoyl-2-oleoylphosphatidylcholine (SOPC) was particularly interesting. Up to about carbon-16, the decrease in orientational order along the chain was similar to that observed for GalCer having an 18 carbon perdeuterated chain under comparable conditions. The decrease in order with position slowed over the last few carbons of the 24-carbon chain, giving rise to a second plateau corresponding to low orientational order. Deuteration of the methyl group and the two nearest methylenes on the 24-carbon chain was used to confirm assignments for the three smallest order parameters in this profile (Lu et al., 1993). The occurrence of a second plateau at low values of the order parameter has previously been observed for long chain guest molecules in lyotropic liquid crystals (Forrest et al., 1980) and in bilayers of potassium palmitate (Tang et al., 1985). Recently, Lewis et al. (1994) have

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

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reported the observation of profiles with a second plateau at low orientational order for highly asymmetric mixed-chain diacyl phosphatidylcholines in the liquid crystalline phase.

Although the observation of a second plateau at low orientational order clearly indicates that the end of a long chain guest molecule at low concentration is very disordered, the question of how the chain is accommodated relative to the host matrix remains to some extent. Given the likelihood of chain backfolding in such a region of disorder, considerable caution must be exercised in the interpretation of mean chain extension as determined from ^{2}H-NMR order parameter measurements. For such systems, questions can also be raised regarding the extent to which the local environment of the long chain departs from the average environment of the bilayer because of the perturbing effects of the long chain on surrounding short chains. One issue that has been of particular interest is the extent to which the acyl chains of the longer component penetrate the opposite side of the bilayer in fluid phase membranes (Morrow et al., 1993).

To gain some insight into the mutual interaction of long and short chain components in a bilayer with chain-length mismatch, we have examined smoothed orientational order parameters in mixtures of dimyristoylphosphatidylcholine (DMPC) and distearoylphosphatidylcholine (DSPC) with one or the other components chain-perdeuterated (DMPC-d_{54} and DSPC-d_{70}, respectively). The acyl chains of DMPC and DSPC differ in length by four methylene groups. The binary mixture has been used extensively as a model in studies of the effect of chain-length mismatch. A number of investigations of this phase diagram have been reviewed in conjunction with two recent ^{2}H-NMR studies of this system (Morrow et al., 1991; Sankaram and Thompson, 1992).

Recently, Sankaram and Thompson (1992) used ^{2}H-NMR to investigate the temperature and composition dependence of bilayer thickness in this system. They measured average chain lengths for DSPC-d_{70} and DMPC-d_{54} in DMPC-DSPC-d_{70} and DMCP-d_{54}-DSPC mixtures, respectively. They assumed that, in the fluid phase, the lengths of DMPC and DSPC at a given temperature were independent of which member of the pair was deuterated. They obtained bilayer thickness by using a weighted average of the two chain lengths corresponding to a particular concentration and temperature. Their calculation of chain lengths from orientational order parameters appears to neglect the possibility of significant backfolding. The calculation of bilayer thickness using such a weighted average is discussed further in an appendix below.

In this work, we have obtained smoothed orientational order parameter profiles (Sternin et al., 1988; Lafleur et al., 1989) in the liquid crystalline phase for DMPC-DSPC-d_{70} mixtures over a range of compositions and for corresponding DMPC-d_{54}-DSPC mixtures. Because of the effect of chain perdeuteration on the main transition temperatures of the pure lipid components, the phase diagrams for these two mixtures are slightly different (Morrow et al., 1991; Sankaram and Thompson, 1992). This raises the question of whether temperature is the appropriate parameter to fix when comparing order parameter profiles for the two components. One alternate way to compare order profiles for the two components is to assume that the two components, in a given mixture, adjust to have the same area per lipid. As pointed out by Nagle (1993), area per lipid molecule in the bilayer is most directly related to the value of the orientational order parameter, S_{CD}, near the headgroup end of the acyl chains. This corresponds to the plateau region of the orientational order parameter profile. A comparison based on this assumption would then involve order parameter profiles for different mixtures and for the pure lipids under conditions that give similar values for the order parameter in the plateau region of the smoothed profile. We have addressed the question of which approach is more valid by comparing the order parameter profiles for one or the other lipid, in a mixture with one component deuterated, to the profile obtained in a mixture with both components deuterated.

MATERIALS AND METHODS

Stearic acid and myristic acid were perdeuterated using the method of Hsiao et al. (1980) and used in the synthesis of DSPC-d_{70} and DMPC-d_{54} following the method of Gupta et al. (1977). The products were purified on a 1.5 m Sephadex LH 20 column. Ordinary DMPC and DSPC were purchased from Sigma Chemical Co. (St. Louis, MO). Mixed samples were prepared by co-dissolving the two lipid components in ethanol. Solvent was removed by rotary evaporation followed by overnight pumping in a dessicator. Samples were prepared with deuterated lipid contents of about 35 mg. Each sample was scraped into an 8 mm NMR tube with a total volume of about 400 μl. The samples were hydrated in an excess of 50 mM phosphate buffer at a pH of 7.0. The spectra examined in this work were selected from larger sets of spectra obtained as mixtures were cooled in one- or two-degree steps from well above the liquidus line for each mixture. In each case, the sample was allowed to equilibrate for about 20 min before collection of transients began.

^{2}H-NMR spectra were obtained using the quadrupole echo sequence (Davis et al., 1976) using a pulse separation of 35 μs and $\pi/2$ pulse lengths ranging from 2 to 3.5 μs. Transients were digitized with an effective dwell time of 4 μs as described previously (Morrow et al., 1993). Other details of the NMR spectrometer have been reported previously (Morrow, 1990). Smoothed order parameter profiles were determined from perdeuterated chain powder spectra using a modification of the approach outlined by Sternin et al. (1988) and Lafleur et al. (1989). The splittings of resolvable doublets were used directly. Resolvable doublets were also used to estimate spectral area per deuteron. This estimate was used to determine the number of deuterons represented by each unresolved portion of the spectrum. The splittings associated with each unresolved portion of the spectrum were then determined by partitioning the integral of spectral intensity across that region. The resulting set of splittings was assigned by assuming a monotonic decay of order along the chain.

RESULTS

The ^{2}H-NMR spectrum of a fluid phase lipid bilayer containing species with perdeuterated acyl chains is a superposition of Pake doublets. Molecules reorienting around axes perpendicular to the applied magnetic field give rise to sharp features split by (Davis, 1983)

$$\Delta\nu_q = \frac{3}{4}\frac{e^2qQ}{h}S_{CD}, \quad (1)$$

where e^2qQ/h is the quadrupole coupling constant and S_{CD} is

the orientational order parameter given by

$$S_{CD} = \tfrac{1}{2}\langle 3\cos^2\theta_{CD} - 1\rangle. \quad (2)$$

The average in Eq. 2 is over orientations of the carbon-deuterium bond, and θ_{CD} is the angle between the carbon-deuterium bond and the bilayer normal. Bloom and coworkers have developed techniques to extract the corresponding oriented sample spectrum from a powder pattern (Bloom et al., 1981; Sternin et al., 1983) and to further obtain a smoothed orientational order parameter profile from the resulting de-Paked spectrum (Sternin et al., 1988; Lafleur et al., 1989) by assuming monotonic decay of order along the acyl chain. Smoothed profiles do not capture the detailed behavior near the headgroup end of the chain (Seelig and Seelig, 1980) but are very useful in characterizing the behavior of the chain as a whole (Lafleur et al., 1990; Morrow and Lu, 1991; Monck et al., 1992).

Smoothed order parameter profiles were obtained for perdeuterated acyl chains of one or the other component in DMPC-DSPC bilayers. The resulting averaging of the *sn*-1 and *sn*-2 profiles should be noted, although no significant effect on the comparisons described below is expected. All spectra were collected above the liquidus boundary of the binary phase diagram. Under such conditions, the bilayer is assumed to exist in a homogeneous liquid-crystalline phase. Spectra from a larger data set have been selected to illustrate specific comparisons between component order parameter profiles.

As will be seen below, for low concentrations of one component, the profiles of the other component are only weakly perturbed from their single-component bilayer shapes. Fig. 1 shows spectra for DMPC-d_{54} at 37°C, DSPC-d_{70} at 57°C, and 4.4 mol% DSPC-d_{70} in DMPC at 42°C. The chain-melting transition temperature for DMPC-d_{54} is slightly higher than 19°C. The transition temperature of the 4.4 mol% DSPC-d_{70} in DMPC mixture is found to be 24.5°C, as would

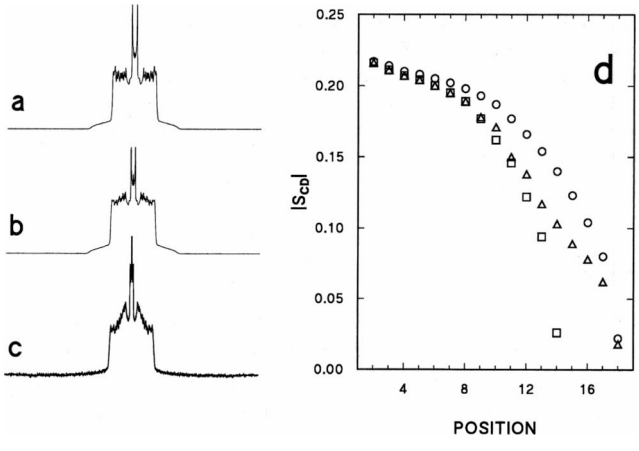

FIGURE 1 (*a*) ^{2}H-NMR spectrum of DMPC-d_{54} in excess water at 37°C. (*b*) ^{2}H-NMR spectrum of DSPC-d_{70} in excess water at 57°C. (*c*) ^{2}H-NMR spectrum of 4.4 mol% DSPC-d_{70} in DMPC at 42°C. (*d*) Smoothed orientational order parameter profiles corresponding to the spectra in *a* (○), *b* (□), and *c* (△).

be expected for nearly pure DMPC with no deuteration. The DMPC-d_{54} profile and the profile for a dilute mixture of DSPC-d_{70} in DMPC were thus both obtained about 18° above the corresponding bilayer transitions. The 5° difference between the temperatures for the DMPC-d_{54} profile and for the 4.4 mol% DSPC-d_{70} in DMPC profile is the expected change in transition temperature that accompanies perdeuteration of the DMPC component. Smoothed order parameter profiles for these spectra are shown in Fig. 1 *d*. The temperature for the DSPC-d_{70} spectrum was selected to yield a smoothed order profile with similar values of the order parameter in the plateau region.

As pointed out by Nagle (1993), the area per lipid molecule in the bilayer should be reflected by the value of the orientational order parameter near the headgroup end of the acyl chains. This roughly corresponds to the plateau region of the smoothed orientational order parameter profile. Based on this argument, the deuterated lipids in the DMPC-d_{54} and DMPC-DSPC-d_{70} bilayers giving rise to the spectra in Fig. 1 have similar areas per lipid at corresponding temperatures above the transition. If it is assumed that the dependence of area per lipid on temperature above the transition is similar for perdeuterated and normal DMPC and if it is assumed that the small concentration of DSPC has a minor influence on the DMPC order parameter profile, as will be shown below, then we can approximate the unknown DMPC profile in the mixture at 42°C by the DMPC-d_{54} profile at 37°C. The comparison in Fig. 1 *d* would then suggest that for diacyl phospholipids having the same headgroup, the minor component in a very dilute binary mixture adopts nearly the same area per lipid as the major component.

Fig. 1 *d* also shows the smoothed order parameter profile for DSPC-d_{70} at a temperature (57°C) selected to give a corresponding plateau value of the order parameter. This is only 6° above the transition temperature for DSPC-d_{70} and is evidence that area per lipid in the fluid phase does not depend only on the separation, in temperature, from the transition. At very low concentrations in the mixture, the plateau order and, by extension, the area per lipid of DSPC-d_{70} is strongly influenced by the surrounding lipids in the bilayer. It is also notable that between carbon-12 and carbon-17, the DSPC-d_{70} profile for the mixture becomes progressively similar to DSPC-d_{70} in the single-component bilayer and progressively different from DMPC-d_{54}. These observations are discussed in more detail below.

We are interested in examining profiles for both components corresponding as closely as possible to the profiles they would simultaneously display in a given mixture. Because the phase diagrams for DMPC-DSPC-d_{70} and DMPC-d_{54}-DSPC mixtures differ slightly (Sankaram and Thompson, 1992), it is necessary to determine an appropriate way to select corresponding spectra with one or the other component deuterated. The comparison illustrated in Fig. 1 relies on the assumption that the areas per lipid for DMPC-d_{54} and proteated DMPC in single-component bilayers depend on temperature above the transition in the same manner. Also implicit in this comparison is the assumption that the transition

is associated with a particular value of the area per lipid of the major component. For the very low DSPC concentration used in Fig. 1, the transition is broadened only slightly, and correlating host and guest spectra based on temperature above the transition seems reasonable. For higher concentrations of DSPC, however, two phases coexist over a wide temperature range (Morrow et al., 1991), and there is no unique transition temperature and no reason to assume a common relationship between temperature and area per lipid for the two components.

We have examined two possible ways of correlating spectra obtained from the alternately deuterated mixtures. If area per lipid for a given component in the fluid binary mixture at a given temperature is not altered by deuteration and is insensitive to deuteration-related changes in the binary phase diagram, then comparing spectra obtained at a given temperature should be appropriate. One alternate possibility is that the two components share a common area per lipid in the mixture. In this case, the appropriate comparison would be between spectra obtained at temperatures for which the plateau values of the orientational order parameters are the same for the two components in mixtures with one or the other component perdeuterated. To test the first possibility independently, we examined DMPC/DSPC mixtures having 50 mol% DSPC with each of the components deuterated separately and with both components deuterated simultaneously.

Fig. 2 shows spectra for DMPC-DSPC-d_{70}, DMPC-d_{54}-DSPC, and DMPC-d_{54}-DSPC-d_{70} at 60°C. It can be seen that at a fixed temperature, there is a small but distinct difference

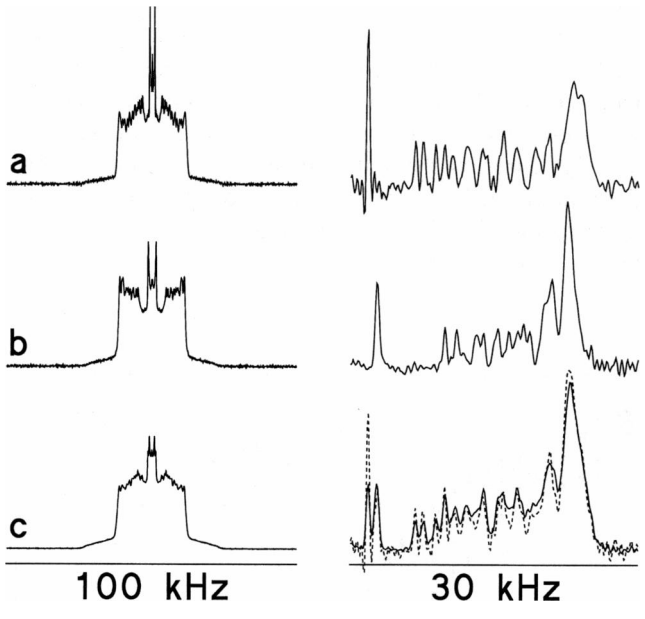

FIGURE 2 ^{2}H-NMR spectra (*left*) and half of the corresponding de-Paked spectra for DMPC-DSPC mixtures at $x_{DSPC} = 0.5$ and $T = 60°C$. (*a*) DMPC-DSPC-d_{70}. (*b*) DMPC-d_{54}-DSPC. (*c*) DMPC-d_{54}-DSPC-d_{70}. The de-Paked spectrum shown as a solid line corresponds to the observed DMPC-d_{54}-DSPC-d_{70} powder pattern. The de-Paked spectrum shown as a dashed line is the weighted sum of de-Paked spectra for DMPC-DSPC-d_{70} and DMPC-d_{54}-DSPC.

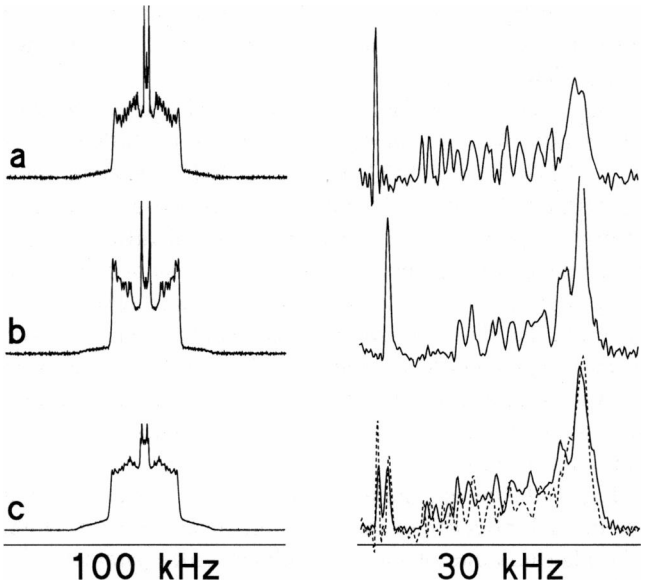

FIGURE 3 ^{2}H-NMR spectra (*left*) and half of the corresponding de-Paked spectra for DMPC-DSPC mixtures at $x_{DSPC} = 0.5$. Temperatures were selected to yield powder patterns with the same maximum splitting. (*a*) DMPC-DSPC-d_{70} at 60°C. (*b*) DMPC-d_{54}-DSPC at 53°C. (*c*) DMPC-d_{54}-DSPC-d_{70} at 56°C. The de-Paked spectrum shown as a solid line corresponds to the observed DMPC-d_{54}-DSPC-d_{70} powder pattern. The de-Paked spectrum shown as a dashed line is the weighted sum of de-Paked spectra for DMPC-DSPC-d_{70} and DMPC-d_{54}-DSPC.

between the maximum quadrupole splittings for the two components in the mixtures with only one component deuterated. This implies a slight difference in the plateau values of the orientational order parameter and suggests that they do not share a common value for the area per lipid. Fig. 2 also shows oriented spectra, obtained by de-Pakeing powder spectra for the mixtures with one or the other or both components perdeuterated. Superimposed on the latter spectrum is a weighted sum of the two single-deuterated component spectra. The sum spectrum displays splittings that correspond closely to those obtained with both components deuterated.

This correspondence is not found if the spectra are selected to have similar values of the orientational order parameter in the plateau. Fig. 3 shows such a comparison. The weighted sum of the two single-deuterated component spectra does not correspond to the spectrum observed with both components deuterated. It seems clear that within this mixture, the two lipid components are not constrained to display equal plateau order parameters and thus, by extension, are not constrained to have equal areas per lipid. This observation initially appears to be inconsistent with the comparison illustrated in Fig. 1. However, it is interesting to note that, for concentrations between 30 mol% DSPC and 75 mol% DSPC, the averages of the liquidus and solidus temperatures for the DMPC-DSPC-d_{70} and DMPC-d_{54}-DSPC phase diagrams are the same to within about 1° (Sankaram and Thompson, 1992). It may be that over a wide range of concentrations, comparing pairs of spectra at fixed temperature is equivalent

to comparing them at the same separation from an effective transition temperature that falls near the center of the two phase region.

Fig. 4 shows smoothed orientational order parameter profiles at 60°C for the deuterated components in DMPC/DSPC-d_{70} and DMPC-d_{54}/DSPC at nominal DSPC concentrations 25, 50, and 75 mol%. Given the relatively gradual change in profile shape with concentration, the small difference between the mixture compositions in Fig. 4 *c* is not considered to be significant. Profiles for DMPC-d_{54} and DSPC-d_{70} at 60°C are also shown for comparison. In light of the preceding discussion, the DSPC-d_{70} and DMPC-d_{54} profiles obtained at a given composition and temperature are assumed to represent simultaneously the states of the two components in the particular mixed bilayer.

Fig. 4 illustrates a number of interesting points regarding the mutual influence of the two components in the mixture. The plateau value of the orientational order parameter for each component in the mixture departs from its value for the corresponding single component bilayer in a concentration-dependent way. For the 1:1 mixture, the disordering of DSPC-d_{70} relative to the pure lipid bilayer is similar in magnitude to the corresponding ordering of DMPC-d_{54}. The areas per lipid for both components tend toward a weighted mean of the two single-component bilayer values. Over the range of DSPC mole fractions between 0.25 and 0.75, however, the difference between the plateau values of the order parameter for the two components is approximately maintained. One simple observation that can thus be made is that there is little difference between the two lipids in terms of the susceptibility of their area per lipid to changes in the average order of the surrounding matrix.

For concentrations below 25 mol% DSPC or above 75 mol% DSPC, the profiles of the major components in the mixtures are already very close to the corresponding single-component bilayer profiles. It is likely that for higher and lower DSPC concentrations, the profile of the major component becomes insensitive to concentration, whereas that of the minor component increasingly approaches the former. This expectation is consistent with the observations illustrated by Fig. 1 provided that the effect of deuteration on the transition temperature is taken into account.

The shapes of the smoothed orientational order parameter profiles display a subtle but interesting concentration dependence. For both components, the largest absolute change in order parameter appears to occur on that portion of the chain beyond the plateau region of the profile. As the concentration of DMPC is reduced, the order in this region of its profile increases at a slightly higher rate than in the plateau region. The result is a stretching of the DMPC plateau with decreasing DMPC concentration. Similarly, as the DSPC concentration is reduced, the order beyond the plateau of its profile decreases more quickly than in the plateau. The result is the appearance of a second "plateau" at relatively low order corresponding to deuterons on carbons 14–17. This type of secondary plateau was recently reported for 24:0 fatty acids on GalCer in SOPC bilayers (Morrow et al., 1993; Lu et al., 1993). It should be noted that the dependence of profile shape on DSPC concentration is relatively weak and apparently continuous.

Although the shape and plateau values of the smoothed order parameter profiles for the two components depend on bilayer composition, the difference in mean chain extension at a given temperature is effectively independent of concentration. Using a model such as that given by Schindler and Seelig (1975) in which back-folding is neglected, the DSPC-d_{70} chain is found to extend about 3.1 Å further than the DMPC-d_{54} chain for all three of the profile pairs shown in Fig. 4.

The effect of bilayer composition on the shape of the smoothed orientational order parameter profiles for the two components can be separated from the effect on plateau order and area per lipid by selecting spectra, for different compositions, having the same value of the order parameter in the plateau region. It should be borne in mind that, based on the preceding discussion, these profiles do not simultaneously represent the states of the two components in a given mixture. Nevertheless, they do give an indication of the manner in which chain-length mismatch is accommodated by the bilayer.

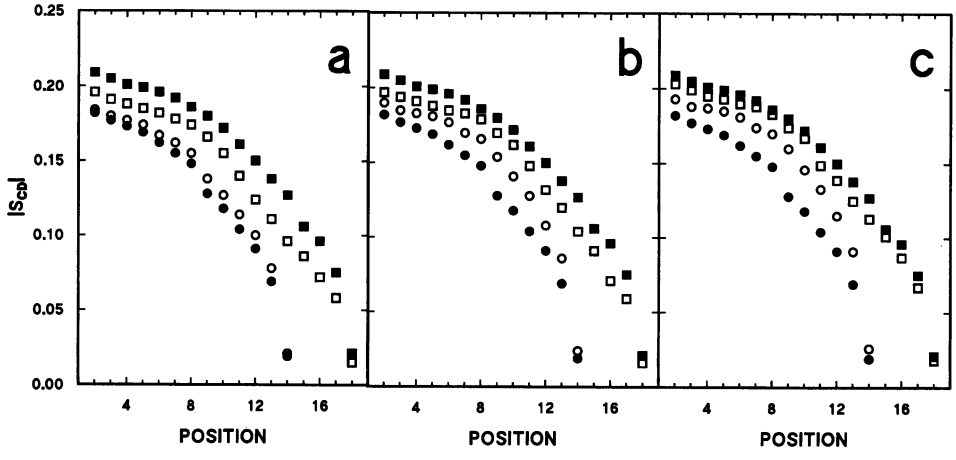

FIGURE 4 Smoothed orientational order parameter profiles at 60°C. (*a*) DSPC-d_{70} (■), DMPC-d_{54} (●), DMPC-DSPC-d_{70} at x_{DSPC} = 0.25 (□), DMPC-d_{54}-DSPC at x_{DSPC} = 0.25 (○). (*b*) DSPC-d_{70} (■), DMPC-d_{54} (●), DMPC-DSPC-d_{70} at x_{DSPC} = 0.50 (□), DMPC-d_{54}-DSPC at x_{DSPC} = 0.50 (○). (*c*) DSPC-d_{70} (■), DMPC-d_{54} (●), DMPC-DSPC-d_{70} at x_{DSPC} = 0.75 (□), DMPC-d_{54}-DSPC at x_{DSPC} = 0.70 (○).

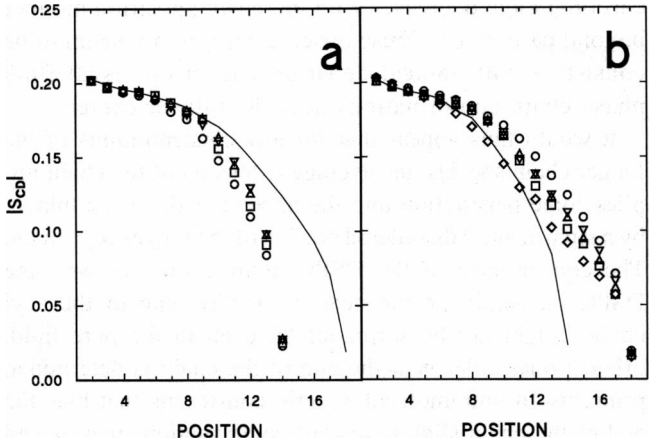

FIGURE 5 Smoothed orientational order parameter profiles at temperatures chosen to yield powder patterns with the same maximum splitting. (*a*) DMPC-d_{54}-DSPC at x_{DSPC} = 0.25 and T = 48°C (□), DMPC-d_{54}-DSPC at x_{DSPC} = 0.50 and T = 51°C (△), DMPC-d_{54}-DSPC at x_{DSPC} = 0.70 and T = 53°C (▽), DMPC-d_{54} at T = 44°C (○). The solid line is drawn through points for DSPC-d_{70} at T = 64°C. (*b*) DMPC-DSPC-d_{70} at x_{DSPC} = 0.044 and T = 50°C (◇), DMPC-DSPC-d_{70} at x_{DSPC} = 0.25 and T = 56°C (□), DMPC-DSPC-d_{70} at x_{DSPC} = 0.50 and T = 58°C (△), DMPC-DSPC-d_{70} at x_{DSPC} = 0.75 and T = 60°C (▽), DSPC-d_{70} at T = 64°C (○). The solid line is drawn through points for DMPC-d_{54} at T = 44°C.

Fig. 5 *a* shows smoothed orientational order parameters for DMPC-d_{54} in DMPC-d_{54}-DSPC mixtures over a range of DSPC mole fractions. The temperatures have been chosen to give the same plateau value for the order parameter. Profiles for DSPC-d_{70} and DMPC-d_{54} in single lipid bilayers are also shown for temperatures giving the same plateau. Fig. 5 *b* shows a similar set of profiles for DSPC-d_{70} in DMPC-DSPC-d_{70} mixtures. It has recently been demonstrated that for a given lipid component under a variety of conditions, the shape of the smoothed orientational order parameter profile is largely determined by the mean order parameter and is insensitive to a variety of local perturbations (Lafleur et al., 1990). If position along the chain is scaled, it is also found that the order parameter profiles for lipids having different chain lengths but the same headgroup are also determined largely by the plateau value of the order parameter (Morrow and Lu, 1991). In Fig. 5, the DMPC-d_{54} and DSPC-d_{70} profiles obtained for the single-lipid bilayers can be superimposed by means of a suitable scaling of position along the chain. The mixture order parameter profiles are bounded by these pure lipid profiles. It is apparent, however, that the profiles for the two components obtained from the mixed bilayers do not satisfy a simple scaling relationship and thus differ in detail from pure lipid profiles corresponding to the same area per lipid.

DISCUSSION

Mean orientational order at a given point on a chain in a mixed bilayer should reflect both intermolecular and intramolecular influences. The composition of the bilayer and the way in which the molecule samples the range of environments in the bilayer will determine an effective ordering potential. The response to a particular ordering potential is determined, in part, by the nature of the molecule. For a given ordering potential, a longer-chain lipid should display more order in the plateau region because constraints on internal motion increase with the length of the chain. This is due, at least in part, to increased hindrance. The observations presented here suggest that there is a simple and continuous dependence of plateau order parameter values on bilayer composition. In each case, the observed mixed-bilayer profile for a given component falls roughly between the single-component bilayer profile for that component and the profile that would be obtained by taking an average, weighted by the bilayer composition, of the two single-component bilayer profiles. It should be noted that the observation of increased ordering with increasing mean chain length implies that the dominant interactions are those between molecules on the same side of the bilayer. Isolated longer-chain molecules on one side of the bilayer might be expected to exert a disordering influence on molecules on the opposite side of the bilayer.

Additional insight into the accommodation of chain-length mismatch can be obtained from a more detailed examination of the smoothed order parameter profile shapes in the mixtures. Within a model such as the one presented by Schindler and Seelig (1975), the mean extension per segment along the bilayer normal of a chain segment depends linearly on the magnitude of the orientational order parameter. Because such a calculation assumes no backfolding of the chain, extensions calculated in this way must be considered to be overestimates of mean chain extension. However, because backfolding will affect both chains to some extent, differences in chain extension should be less sensitive to its presence. At 60°C, for concentrations between 25 mol% DSPC and 75 mol% DSPC, the mean extensions of the two chains differ by about 3.1 Å with effectively no dependence on composition. Between carbons 2 and 12, the average extension of each DSPC chain segment is greater than the corresponding DMPC chain segment. It is likely that the probability for chain backfolding is non-negligible only for the last few segments of a saturated acyl chain (Nagle, 1993; Meraldi and Schlitter, 1981). It would thus appear that, on average, the DSPC chains extend significantly further into the bilayer than the DMPC chains. As has been emphasized by Sankaram and Thompson (1992), however, length measurements by NMR are averages and do not necessarily indicate local conditions in the bilayer. It is important to bear this point in mind, particularly when considering profiles for mixtures with a small concentration of one or the other component.

Comparisons of average chain extension for the two components are not sufficient to yield a unique picture of how chain length mismatch is accommodated in the bilayer. Consider, for example, a mixture in which the longer-chain molecules are present at low concentration. Local ordering or disordering of the higher concentration, shorter chain species

in the neighborhood of longer chain molecules might influence the extent to which the extra length of the longer chain penetrates the opposite side of the bilayer by locally shifting the interface between opposite sides away from the geometric bilayer center. Because of the averaging inherent in chain length measurement by NMR, the presence or absence of such local modulation of the interface between opposite sides of the bilayer might not be directly apparent from orientational order parameter measurements on the higher concentration component. However, the DSPC-d_{70} smoothed profiles for lower DSPC concentrations provide clues regarding the local interface between opposite sides of the bilayer. The largest disordering of a DSPC chain, relative to the state of the chain in a pure DSPC bilayer, is likely to occur near the methyl groups of adjacent DMPC molecules. Within the mixtures, the greatest departure of the DSPC order parameter from the corresponding value in the pure DSPC bilayer occurs over a broad range of positions centered on carbon-14 and is thus roughly coincident with the mean depth of the DMPC methyl groups. This observation suggests that the depths of DMPC methyl groups near DSPC molecules are close to the mean methyl group depth obtained by averaging over the entire DMPC population. Isolated DSPC molecules appear to cause little local modulation of the interface between opposite sides of the bilayer. A simple way to summarize this argument is that for low DSPC concentrations, the DSPC profile reports on a local environment that "travels" with the DSPC molecule, whereas the DMPC profile reflects an average over the entire population. The observation that the largest disordering of the DSPC chain relative to pure DSPC occurs at a depth coincident with the average location of the DMPC methyls indicates that the extension of DMPC chains in the neighborhood of the DSPC molecule does not depart appreciably from the mean DMPC extension.

The preceding argument supports the suggestion that, on average, the last four carbons of the DSPC chain extend more deeply into the bilayer than the average depth of the methyl groups on adjacent DMPC molecules. However, because of the increased possibility of backfolding near the methyl terminus of the DSPC chains, chain extensions near the end of the DSPC chain, calculated from order parameters, do not reliably indicate the extent to which, on average, the chain penetrates the opposite bilayer. For example, when examined in isolation, the occurrence of a second plateau at low values of the orientational order parameter for low concentrations of the long-chain component might be consistent with the last segments of the longer chains forming a very disordered fluid region effectively between the two sides of the bilayer (Forrest et al., 1980; Tang et al., 1985; Morrow et al., 1993). However, when the long-chain profiles for a range of concentrations are compared with the corresponding single-component bilayer profile, it is apparent that the orientational orders of the last few segments on the DSPC chain vary only gradually with concentration and, even at low concentration, depart only modestly from the single-component bilayer profile. For DSPC at low concentration, the departure from the corresponding pure DSPC profile is largest near carbon-14 and decreases with increasing position number beyond carbon-14. These observations do not seem to be consistent with radical departures from the usual fluid phase chain conformations near the bilayer center.

It would thus appear that for low concentrations of the longer chain species, the average extension of the chain implies some penetration into the opposite side of the bilayer by a significantly disordered portion of the longer acyl chain. The convergence of the DSPC mixture profiles with the DSPC pure-lipid profile near the methyl end of the acyl chains might not be surprising if, even in the pure lipid, orientational order near the end of the chain is determined primarily by intramolecular steric constraints that bias the end of the chain slightly toward conformations that extend the chain away from the bilayer surface on average. It does not appear that the methyl ends of the DSPC acyl chains experience any significant ordering influence from DMPC chains on the opposite side of the bilayer. Similarly, the DMPC acyl chain shows no evidence of extra disordering over the last few carbons where interaction with DSPC acyl chains originating on the opposite side of the bilayer might be present. The situation at the bilayer center for low concentrations of DSPC might thus be best represented as an intermingling of disordered and weakly interacting chain ends. In a study of long chain guest molecules dissolved in lyotropic liquid crystals, the observation of a low order parameter plateau in the profile was interpreted in terms of a central disordered region rather than insertion into the opposite side of the bilayer (Forrest et al., 1980). In another study of guest and host order parameter profiles for fatty acids of various lengths dissolved in potassium palmitate, profiles with secondary plateaus at low values of the order parameter were interpreted as being consistent either with random curling of the excess chain length or rapid exchange between positions lying in the midplane region and positions involving penetration into the opposite side of the bilayer (Beckmann et al., 1980; Tang et al., 1985). For some of the profiles shown in this earlier work, disordering in the second plateau region was more extreme than that observed even for the lowest DSPC concentration in the present work. If, as the results presented here suggest, the plateau at low order, seen for low concentrations of the longer chain component, can be thought of as arising primarily from a reduction of order around carbon-14 of the long chain, the explanation involving rapid exchange between penetrating and nonpenetrating conformations seems to be more appropriate. Again, however, the highly disordered nature of the end of the long chain at low concentrations must be emphasized. The term interdigitation has been most widely used to describe situations in ordered phases of bilayers with significant chain-length mismatch. If it is to be applied at all to fluid phase mixtures such as described here, some care should be taken to avoid the implication of strong interaction between opposite sides of the bilayer. This interpretation is largely consistent with that given by Lewis et al. (1994) who, in a recent study of highly asymmetric mixed-chain phospholipids, attributed the

appearance of order profiles with plateaus at low order parameter to a statistical form of interdigitation for which ends of the long chains sample environments on both sides of the bilayer midplane.

It is interesting to consider how bilayer thickness depends on bilayer composition in light of these observations. Using the profiles of Fig. 4 and a calculation based on the Schindler and Seelig (1975) formalism, the mean extensions of the DMPC and DSPC chains are both found to increase by about 0.2 Å from 25 to 75 mol% DSPC at 60°C despite the changes in profile shape and plateau order parameter. On the other hand, the thickness of one side of the single-component DSPC-d_{70} bilayer at this temperature is roughly 3 Å greater than that of the DMPC-d_{54} bilayer based on the profiles observed here. The rather weak dependence of profile shape on concentration displayed in Fig. 4 suggests that the change in bilayer thickness must be continuous and gradual. As shown in the appendix below, an internally self-consistent treatment of the model presented by Sankaram and Thompson (1992) yields a bilayer thickness that is a weighted mean of the contributions from the two components. The effectively linear dependence of bilayer thickness on concentration arising from this expression would be consistent with the composition dependence of the profiles presented here. It should be noted that the result presented in the Appendix is based on the assumption that the bilayer thickness is given by a weighted average of like and unlike pair thicknesses and thus does not represent a departure from the physical picture underlying the Sankaram and Thompson model.

These results thus seem to suggest a change in bilayer thickness with composition that is nearly an order of magnitude greater than the change in mean extension of either of the two components. Such an observation can only be understood if the bilayer center is disordered to the extent that the ends of different acyl chains can freely interpenetrate the opposite side of the bilayer constrained only by a requirement to maintain hydrocarbon density at the value necessary to support the bilayer. Such a situation might exist if near the bilayer center, chain order were more dependent on intramolecular effects than on intermolecular effects. Changes in the bilayer center with composition might then be pictured as follows. For a single-component DSPC bilayer, the ends of the DSPC acyl chains are very disordered with the mean positions of the methyl groups close to the bilayer midplane. As the DMPC concentration is increased, the disordered ends of the DSPC chains interpenetrate to the degree necessary to maintain the hydrocarbon density at the bilayer midplane. This is accompanied by a slight disordering of the DSPC acyl chains but effectively no change in their mean extension. For a 1:1 mixture of the components, the interface between opposite sides of the bilayer is presumably diffuse. The DSPC acyl chains are slightly disordered, and the DMPC chains slightly ordered relative to the corresponding single-component bilayers. Again, the degree of interpenetration is presumably that necessary to approximately maintain the hydrocarbon density. At low DSPC concentrations, DMPC methyl groups have mean locations close to the bilayer midplane and provide the necessary hydrocarbon density to support the bilayer. The small concentration of DSPC chain ends extend through this region with only slightly less order than in predominantly DSPC bilayers. The rest of the DSPC acyl chain is slightly less ordered than in the single-component bilayer but, again, the change in extension is relatively small.

CONCLUSIONS

Smoothed orientational order parameter profiles of DMPC and DSPC acyl chains in fluid phase binary mixtures have been obtained for a range of bilayer compositions. Order parameter values in the plateau region are found to increase with increasing DSPC concentration for both components. The magnitude of the response is similar for the two components. Shapes of the smoothed profiles for both components are also found to depend on bilayer composition. At low DSPC concentration, the region of the DSPC profile near carbon-14 is depressed relative to the corresponding single-component bilayer profile for DSPC. At high DSPC concentration, the DMPC plateau is stretched relative to the corresponding single-component bilayer profile. At low DSPC concentration, carbon-14 of the DSPC acyl chain appears to coincide roughly with DMPC methyl groups. The last few methylene groups of the DSPC acyl chains are highly disordered but do appear to penetrate, on average, across the bilayer midplane.

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APPENDIX

In calculating an average thickness for DMPC-DSPC bilayers, Sankaram and Thompson (1992) consider the contribution to the thickness from pairs of the higher concentration lipid and from DMPC-DSPC pairs. They do not allow for pairs of the lower concentration molecule. They use x to denote the concentration of DSPC in the bilayer.

For $x < 0.5$, they give the thickness of the bilayer as

$$d = (1 - 2x)d_0 + 2xd_{1:1}, \tag{A1}$$

where d_0 is the contribution from DMPC-DMPC pairs and $d_{1:1}$ is the contribution from DMPC-DSPC pairs. It appears, though, that their expression for d_0 is based on an assumption which is inconsistent with the assumptions inherent in Eq. A1. To see this, we rearrange their expression for d_0 to obtain an expression for the average length, $\langle L \rangle_x^{2\text{-}14}$, of the myristoyl chain in the mixture of concentration x. Their expression yields

$$\langle L \rangle_x^{2\text{-}14} = (1 - x)\frac{d_0}{2} + x\langle L \rangle_{x=0.5}^{2\text{-}14} \tag{A2}$$

where $\langle L \rangle_{x=0.5}^{2\text{-}14}$ is the mean length of the myristoyl chain in a 1:1 mixture of DMPC and DSPC. In Eq. A2, there is an implicit assumption that the probability for one or the other species on one side of the bilayer to be associated with a given DMPC molecule on the other side is given simply by the mole

fraction of that species. This assumption, however, is not consistent with the assumption, inherent in Eq. A1, that pairs of the lower concentration lipid can be neglected. Ruling out such pairs puts a constraint on the number of DMPC molecules available for DMPC-DMPC pairs. Eq. A2 thus overestimates the weighting of d_0.

We can obtain a consistent expression for d_0 by considering the fraction of DMPC involved in DMPC-DMPC pairs and the fraction involved in DMPC-DSPC pairs. If no DSPC-DSPC pairs are allowed for $x < 0.5$, then the number of DMPC molecules in DMPC-DSPC pairs will equal the number of DSPC molecules. The fraction of DMPC involved in DMPC-DSPC pairs will thus be

$$\frac{n_{\mathrm{DSPC}}}{n_{\mathrm{DMPC}}} = \frac{x}{1-x}, \tag{A3}$$

where n_{DSPC} and n_{DMPC} are the numbers of molecules of each species in the bilayer. The fraction of DMPC involved in DMPC-DMPC pairs will be

$$1 - \frac{n_{\mathrm{DSPC}}}{n_{\mathrm{DMPC}}} = \frac{1-2x}{1-x}. \tag{A4}$$

Using these weighting factors in the expression for $\langle L\rangle_x^{2\text{-}14}$ and solving for d_0 yields

$$d_0 = \frac{2}{1-2x}\left((1-x)\langle L\rangle_x^{2\text{-}14} - x\langle L\rangle_{x=0.5}^{2\text{-}14}\right), \tag{A5}$$

for $x < 0.5$. L'hôpital's rule can be used to show that at $x = 0.5$, Eq. A5 yields $d_0 = 2\langle L\rangle_{x=0.5}^{2\text{-}14}$.

When this expression is used in Eq. A1, the resulting expression for d is

$$d = 2(1-x)\langle L\rangle_x^{2\text{-}14} + 2x\langle L\rangle_x^{2\text{-}18} \tag{A6}$$

where

$$\langle L\rangle_x^{2\text{-}18}$$

is the average length of the stearoyl chain in the mixture of concentration x. It is interesting that the dependence on $\langle L\rangle_{x=0.5}^{2\text{-}14}$ seen in Eq. 12 of Sankaram and Thompson's work disappears and the bilayer thickness becomes a simple weighted average of the two chain lengths. It should be noted that the underlying physical picture of the Sankaram and Thompson (1992) model, as represented schematically in Fig. 8 of their paper, is retained in this treatment. The use of Eq. A1, which is equivalent to Eq. 10 in Sankaram and Thompson (1992), assumes that the bilayer thickness is given by a weighted average of like and unlike pair thicknesses, d_0 and $d_{1:1}$, respectively.

For $x > 0.5$, a similar analysis yields

$$d'_0 = \frac{2}{2x-1}\left(x\langle L\rangle_x^{2\text{-}18} - (1-x)\langle L\rangle_{x=0.5}^{2\text{-}18}\right). \tag{A7}$$

It should be noted that Eq. 11 of Sankaram and Thompson appears to have x in place of $(1-x)$. When this is corrected and Eq. A7 is used for d'_0, the expression for d when $x > 0.5$ again reduces to Eq. A6.

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