Studies of Highly Asymmetric Mixed-Chain Diacyl Phosphatidylcholines that Form Mixed-Interdigitated Gel Phases: Fourier Transform Infrared and ^{2}H NMR Spectroscopic Studies of Hydrocarbon Chain Conformation and Orientational Order in the Liquid-Crystalline State

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ABSTRACT Hydrocarbon chain conformational and orientational order in liquid-crystalline bilayers of the highly chain-asymmetric 1-*O*-eicosanoyl, 2-*O*-dodecanoyl and 1-*O*-decanoyl, 2-*O*-docosanoyl phosphatidylcholines were studied by Fourier transform infrared (FTIR) and deuterium nuclear magnetic resonance (^{2}H-NMR) spectroscopy, respectively, and compared with appropriate symmetric-chain phosphatidylcholines at comparable reduced temperatures. FTIR spectroscopy indicates that these two asymmetric-chain phospholipids contain a slightly greater number of kink, a considerably larger number of double-gauche, but a somewhat smaller number of end-gauche conformers than does dipalmitoylphosphatidylcholine, a symmetric-chain phospholipid having the same total number of carbon atoms in its hydrocarbon chains. Moreover, the asymmetric-chain phospholipids also contain a larger total number of gauche conformers, suggesting that their hydrocarbon chains are more disordered overall than are those of dipalmitoylphosphatidylcholine. ^{2}H-NMR studies of the specifically chain-perdeuterated analogs of these asymmetric-chain lipids reveal that the orientational order parameter profiles of their shorter and longer chains differ both qualitatively and quantitatively, regardless of whether they are esterified at the *sn*1- or *sn*2 positions of the glycerol molecule. The longer hydrocarbon chains exhibit unusual orientational order profiles in which the order gradient is steepest in the middle of the chain and relatively shallower in regions adjacent to the carboxyl and methyl termini, whereas the short hydrocarbon chains exhibit orientational order profiles typical of those commonly observed with conventional symmetric chain lipids. When compared at equivalent depths in the bilayer, the shorter hydrocarbon chains of the asymmetric-chain lipids are more orientationally disordered than are their longer chain counterparts. At comparable reduced temperatures, the shorter and longer chains of the asymmetric-chain lipids are more orientationally disordered than those of appropriate short and long symmetric-chain lipids, but the chain-averaged orientational order of the symmetric-chain lipid decreases more sharply with increases in temperature than does that of the comparable chain of the asymmetric-chain species. Moreover, the order plateau regions adjacent to the carboxyl groups of the longer chains of the asymmetric-chain phosphatidylcholines are shorter than those of symmetric-chain lipids of comparable hydrocarbon chain length. Overall, the data indicate that the conformational and orientational order in the liquid-crystalline states of these highly asymmetric-chain lipids differ significantly from those of comparable symmetric-chain lipids. Also, the unusual shape of the orientational order profile of the longer chains of the former is attributed to interaction between the methyl termini regions of the long chains with hydrocarbon chains in opposing monolayers. The latter suggests that some form of hydrocarbon chain interdigitation exists in liquid-crystalline bilayers of these highly asymmetric-chain lipids.

INTRODUCTION

Phosphatidylcholines (PCs) in which different n-saturated fatty acyl chains are esterified at the *sn*1 and *sn*2 positions of the glycerol backbone have been intensively studied as models of the more complex naturally occurring mixed-chain glycerolipids (for reviews, see Huang and Mason, 1986; Huang, 1990). Studies of these asymmetric mixed-chain lipids have identified a structurally interesting class of PCs for which the effective length[1] of one acyl chain is approximately twice the length of the other (McIntosh et al., 1984; Hui et al., 1984; Huang and Mason, 1986; Xu and Huang, 1987; Xu et al., 1987; Mattai et al., 1987; Wong and Huang, 1989; Huang, 1990; Shah et al., 1990; Slater et al., 1992; Zhu and Caffrey, 1993; Lewis and McElhaney, 1993; Lewis et al., 1994). The thermodynamic properties of hydrated bilayers composed of these highly asymmetric mixed-chain PCs dif-

[1] Acyl chain length after correction for the conformational inequivalence between the *sn*1 and *sn*2 fatty acyl chains.

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Abbreviations used: DSC, differential scanning calorimetry; MAS-NMR, magic angle spinning nuclear magnetic resonance; PC, phosphatidylcholine (1-*O*-acyl, 2-*O*-acyl, *sn*-glycero 3-phosphorylcholine); 16:16 PC, 1-*O*-palmitoyl, 2-*O*-palmitoyl phosphatidylcholine (dipalmitoyl phosphatidylcholine); 12:12 PC, 1-*O*-dodecanoyl, 2-*O*-dodecanoyl phosphatidylcholine; 20:20 PC, 1-*O*-eicosanoyl, 2-*O*-eicosanoyl phosphatidylcholine; 18:10 PC, 1-*O*-octadecanoyl, 2-*O*-decanoyl phosphatidylcholine; 10:22 PC, 1-*O*-decanoyl, 2-*O*-docosanoyl phosphatidylcholine; 20:12 PC, 1-*O*-eicosanoyl, 2-*O*-dodecanoyl phosphatidylcholine; 22:12 PC, 1-*O*-docosanoyl, 2-*O*-dodecanoyl phosphatidylcholine; Lyso-PC, 1-*O*-acyl lysophosphatidylcholine; FTIR, Fourier transform infrared; T_m, gel/liquid-crystalline phase transition temperature; S_{CD}, CD_2 orientational order parameter; $\langle S \rangle$, chain-averaged CD_2 order parameter.

fer from those of symmetric-chain PCs and other n-saturated mixed-chain PCs for which the hydrocarbon chain asymmetry is less pronounced (see Huang and Mason 1986; Huang, 1990, 1991; Marsh, 1992). Also, recent DSC, x-ray diffraction, and FTIR spectroscopic studies have shown that these lipids are quite unusual in that the thermotropic phase behavior observed upon cooling from the liquid-crystalline phase is affected by the thermal history of the sample in the gel phase (Lewis et al., 1994). The unusual thermotropic phase behavior exhibited by these highly asymmetric PC bilayers has been correlated with their tendency to form so-called mixed-interdigitated gel phases when cooled to temperatures below T_m (Hui et al., 1984; McIntosh et al., 1984). In this particular form of gel-phase packing, the lipid bilayer is organized as an assembly of dimeric units in which two PC molecules are arranged with their long chains fully interdigitated across the lipid bilayer and with the methyl termini of their short chains opposed at the center of the bilayer (see Hui et al., 1984; McIntosh et al., 1984; Shah et al., 1990, Zhu and Caffrey, 1993; Lewis et al., 1994).

Most previous studies of this unusual class of PCs have focused on their thermotropic phase behavior (Huang and Mason, 1986; Xu et al., 1987; Xu and Huang 1987; Mattai et al., 1987; Shah et al., 1990; Huang, 1990; Slater et al., 1992; Lewis et al., 1994), their miscibility with other species of PCs (Xu et al., 1987; Lin and Huang, 1988; Ali et al., 1989; Sisk et al., 1990; Bultmann et al., 1991; Slater et al., 1992; Sisk and Huang, 1992), and the structure of their mixed-interdigitated gel phases (Hui et al., 1984; McIntosh et al., 1984; Wong and Huang, 1989; Shah et al., 1990; Zhu and Caffrey, 1993; Lewis and McElhaney, 1993; Lewis et al., 1994). Indeed, an internally consistent and fairly detailed structural picture of their mixed interdigitated gel phases has emerged from such studies (for a recent discussion, see Lewis and McElhaney, 1993). In contrast, relatively little is currently known about structural and dynamic properties of the liquid-crystalline state of these lipids. However, a MAS ^{1}H- and ^{13}C-NMR study indicates that there are more restrictions on hydrocarbon chain segmental reorientation in the liquid-crystalline phase of 18:10 PC than there are in liquid-crystalline 16:16 PC bilayers (Halladay et al., 1990), and a recent x-ray diffraction study of 22:12 PC (Zhu and Caffrey, 1993) indicates that this lipid forms an unusually "thin" bilayer at temperatures above its T_m. Both of these observations suggest that some interdigitation of the hydrocarbon chains of these highly asymmetric-chain PCs persists upon melting of the mixed-interdigitated gel phase. To examine this possibility and to extend these previous studies, we have carried out FTIR and ^{2}H-NMR spectroscopic studies of hydrocarbon chain conformational and orientational order in the liquid-crystalline phases of two highly chain-asymmetric PCs and have attempted to study the behavior of the short and long hydrocarbon chains individually. The particular lipids we have chosen for examination are 10:22 PC and 20:12 PC, a pair of asymmetric-chain lipids that have been studied previously (see Xu et al. (1987), Lewis and McElhaney (1993), Lewis et al. (1994), and references cited therein). These PCs are ideally suited for studies of this kind because the effective lengths of the two short and the two long chains in this pair of lipids are comparable because of the conformational inequivalence of the *sn*1 and *sn*2 fatty acyl groups (see Hauser et al., 1988). Moreover, for each lipid, the short and long hydrocarbon chains together contain the same number of hydrocarbon atoms as do the hydrocarbon chains of 16:16 PC, one of the most extensively studied of the symmetric-chain phospholipids.

MATERIALS AND METHODS

The unlabeled mixed-chain PCs and the specifically chain-perdeuterated PCs used in this study were synthesized by the acylation of appropriate lyso-PCs with an appropriate fatty acid anhydride and 4-pyrrolidino pyridine as a catalyst (see Lewis and McElhaney (1992) for full experimental details). The unlabeled symmetric-chain PCs were synthesized by methods previously used in this laboratory (Lewis et al., 1987). The PCs were subsequently purified by the chromatographic procedures described by Lewis and McElhaney (1985). The unlabeled lyso-PCs were obtained commercially (Avanti Polar Lipids, Alabaster, AL), whereas the chain-perdeuterated lyso-PCs were synthesized from their respective diacyl PCs by previously published methods (Mason et al., 1980). The isotopically labeled diacyl PCs used to synthesize the lyso PCs were themselves synthesized from the appropriate labeled fatty acids and purified by the same methods used to purify unlabeled symmetric-chain PCs (see above). Decanoic acid (d_{19}), dodecanoic acid (d_{23}), and eicosanoic acid (d_{39}) were obtained from commercial sources (MSD Isotopes, Montreal, Quebec, Canada) whereas docosanoic acid (d_{43}) was synthesized by deuterium-exchange methods (Hsiao et al., 1974), purified by column chromatography, and recrystallized from hexane.

For the infrared spectroscopic experiments, 10 mg of the dried lipid sample was dispersed in 50 μl of water by vigorously vortexing at temperatures well above the T_m of the lipid. The thick paste was then squeezed between the BaF2 windows of a heatable liquid cell (equipped with a Teflon spacer) to form a 10-micron film. Once mounted in the sample holder of the instrument, the sample temperature could be controlled (between -20 and 90°C) by an external, computer-controlled circulating water bath. The infrared spectra were recorded with a Digilab FTS-40 infrared spectrometer using the acquisition parameters previously described by Mantsch et al. (1985). The spectra obtained were analyzed using software supplied by Digilab Inc. (Cambridge, MA) and other computer programs obtained from the National Research Council of Canada. In this study, the primary focus is the conformationally sensitive CH_2 wagging absorption bands near 1367, 1355, and 1341 cm^{-1}. The general procedures used to interpret the integrated intensities of these bands in terms of conformer populations are similar to those used by Casal and McElhaney (1990). Specifically, background absorption attributable to water and water vapor was first subtracted from the original spectrum and a baseline for the 1320 to 1400 cm^{-1} region of the spectrum was constructed by cubic spine interpolation and itself subtracted. Next, Fourier deconvolution methods (Kauppinen et al., 1981) were used to obtain accurate estimates of the peak frequencies of the absorption bands present, and the characteristics of the individual absorption bands were estimated by curve-fitting to Gaussian-Lorentzian functions. Typically, best-fit band parameters were obtained with band shapes corresponding to a Gaussian proportion of some 70%. The integrated intensities of the bands of interest (1367, 1355, and 1341 cm^{-1}) were normalized against that of the CH_3 symmetric bending absorption band at 1378 cm^{-1}, and the normalized integrated intensities were converted to conformer concentrations using the conversion factors obtained from Holler and Callis (1989).

The ^{2}H-NMR spectroscopic experiments were performed with a home-built instrument operating at 46.175 MHz for ^{2}H. ^{2}H-NMR spectra of the specifically chain-perdeuterated lipid samples were recorded using the data acquisition protocol reported by Monck et al. (1992). Typically, spectra were acquired by the co-addition of 12,000–40,000 transients using the quadrupolar echo pulse sequence reported by Davis (1983) with a 4 μs, 90° pulse length, a 50 μs interpulse spacing, a 30.1 μs ring down delay, and a

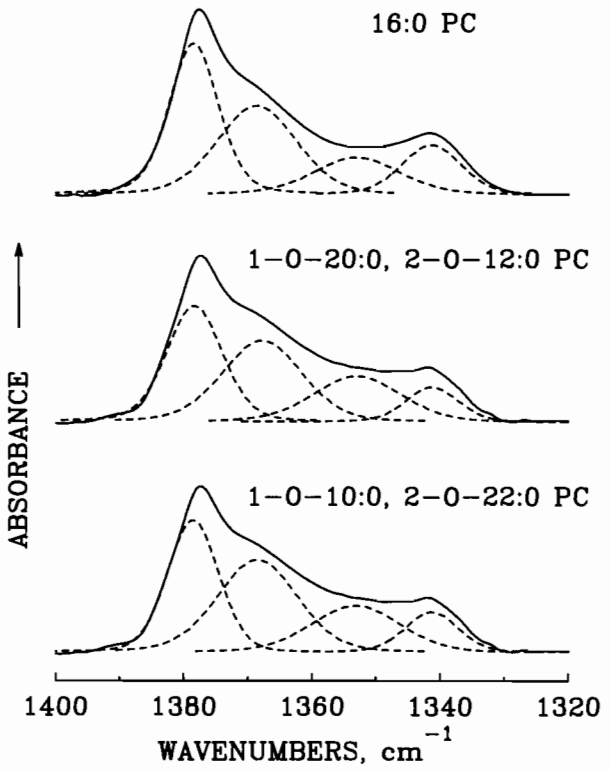

FIGURE 1 The CH_2 region of the infrared spectra in the liquid-crystalline phases of 16:16 PC (*top*), 20:12 PC (*middle*) and 10:22 PC (*bottom*). The absorbance spectra shown have been corrected for solvent and water vapor absorption, and baseline-corrected by cubic spline interpolation. The solid lines indicate the contours of the absorption bands observed, and the dashed lines indicate estimates of the component bands as derived by a combination of Fourier deconvolution and curve fitting.

300 ms recycle delay. The procedures for "dePaking" the spectra obtained and for the derivation of ^2H order profiles from the dePaked spectra were the same as reported previously (Lafleur et al., 1989; Monck et al., 1992). We realize that the ^2H orientational order parameter profiles produced by this methodology differ somewhat from those produced from studies of a series of specifically deuterated fatty acid derivatives (for examples, see Lafleur et al., 1989; Sankaram and Marsh, 1989). Specifically, the plateau regions of ^2H orientational order parameter profiles obtained from the dePaked spectra of chain-perdeuterated derivatives are gently curving rather than flat, and the assigned S_{cd} values at and near to C2 of *sn*2 fatty acyl chains are overestimated. Nevertheless, the procedure developed by Lafleur et al. (1989) accurately reproduces the quadrupolar splittings of the methyl terminal half of the fatty acyl chain and produces comparable chain-averaged order parameter values. In this study, deuterium order parameters are only used for comparative purposes and, more importantly, differences between the order parameter values of the short and long chains of the asymmetric chain lipids, and between the chain-symmetric and chain-asymmetric lipids are manifest primarily in the methyl terminal half of the long chains (see Results). Thus, the picture of hydrocarbon chain order provided by the procedure of Lafleur et al. (1989) should be adequate for the purposes of this study.

RESULTS

FTIR spectroscopic studies of hydrocarbon chain conformation

The 1400–1320 cm^{-1} region of the infrared spectra of unlabeled samples of 16:16 PC, 10:22 PC, and 20:12 PC is illustrated in Fig. 1. The spectra shown were all obtained in the liquid-crystalline state at comparable reduced temperatures (i.e., T_m + 6°C). In this region of the infrared spectrum, compounds containing conformationally disordered polymethylene chains exhibit conformationally sensitive absorption bands near 1367, 1355, and 1341 cm^{-1}. These bands have been assigned to the wagging vibrations of methylene segments existing in the kink, double-gauche, and end-gauche conformations, respectively (Snyder, 1967; Maroncelli et al., 1982). This region of the infrared spectrum also contains an absorption band at 1378 cm^{-1}, which has been assigned to the symmetric bending vibrations of the terminal methyl groups (see Mendelsohn and Mantsch (1986) and references cited therein). The latter band is conformationally insensitive and, thus, can serve as an internal standard against which the integrated intensities of the other three bands can be normalized (Holler and Callis, 1989; Casal and McElhaney, 1990). The normalized band intensities so obtained can be used to estimate the concentrations of the various conformers present (Holler and Callis, 1989; Casal and McElhaney, 1990). The conformer concentrations (per 16 carbon atoms) estimated from the spectra shown in Fig. 1 are listed in Table 1. The conformer distribution in the liquid-crystalline states of the two asymmetric-chain compounds studied (10:22 PC and 20:12 PC) are similar but different from that of a symmetric-chain PC containing the same number of carbon atoms (16:16 PC). Specifically, the overall concentration of gauche bonds in the liquid-crystalline states of the asymmetric-chain compounds is slightly higher than that of the comparable symmetric-chain lipid, suggesting a greater degree of conformational disorder in the hydrocarbon chains of the asymmetric-chain PCs. The principal reasons for this observation are the presence of higher concentrations of double-gauche conformers and lower concentrations of end-gauche conformers in the hydrocarbon chains of the asymmetric-chain PCs. Given the structures of the highly asymmetric-chain PCs studied, one could suggest that the higher content of double-gauche conformers reflects a greater degree of conformational freedom in the longer acyl chain, whereas the relatively lower content of end-gauche conformers reflects the greater constraints on the conformational freedom of the shorter acyl chain. In principle, this suggestion could be tested by individually determining the conformer distribution in the short and in the long chains of specifically chain-perdeuterated analogs of these lipids. However, we found that the CH_2 wagging regions of the infrared spectra of specifically chain-perdeuterated ana-

TABLE 1 Conformer concentrations in the liquid-crystalline states of 16:16 PC, 20:12 PC, and 10:22 PC

Conformer	16:16 PC	20:12 PC	10:22 PC
Kinks (1367 cm^{-1})	1.07	1.11	1.18
End *gauche* (1341 cm^{-1})	0.9	0.64	0.7
Double *gauche* (1355 cm^{-1})	0.58	0.84	0.82
Total *gauche*	4.2	4.5	4.7

Conformer concentrations are expressed in units per 16 carbon atoms. The absorption maxima of the individual bands are indicated in parentheses.

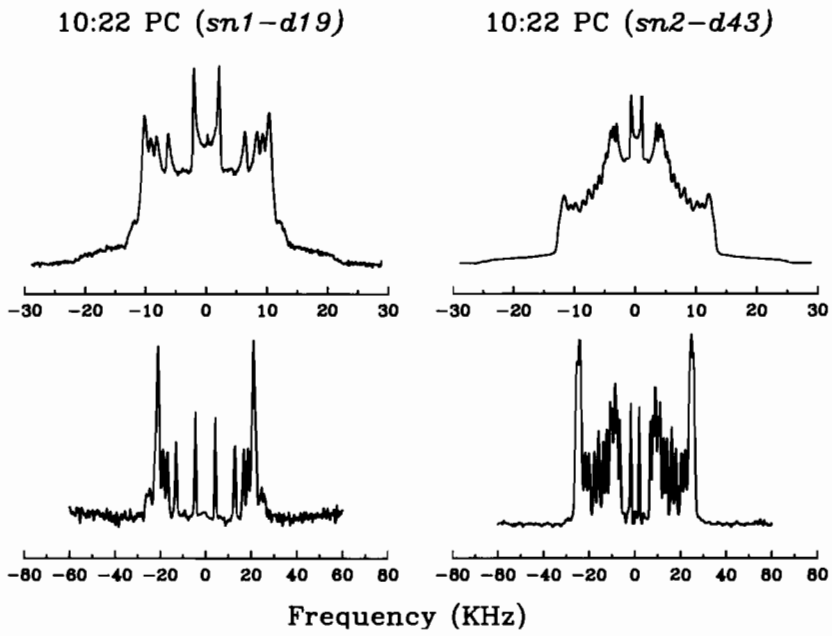

FIGURE 2 ^{2}H-NMR spectra of the two chain perdeuterated analogs of 10:22 PC. Data are presented for *sn*1-d_{19} 10:22 PC (*left* panel, short-chain perdeuterated) and *sn*2-d_{43} 10:22 PC (*right* panel, long-chain perdeuterated). The spectra recorded are shown in the top panel, and the corresponding "dePaked" spectra shown in the bottom panel.

logs of these lipids contain other absorption bands that are of comparable or greater integrated intensity and that overlap one or more of the four bands described above. Moreover, the frequencies and relative intensities of these bands were not the same for each of the specifically chain-perdeuterated PCs studied. We suspect that these "extraneous" absorption bands are the result of incomplete deuteration of the labeled fatty acyl chains and probably arise from vibrational modes associated with the CHD group. With the unexpected appearance of these extraneous bands, the accuracy with which the integrated intensities of the absorption bands of interest could be measured was severely diminished, and our ability to estimate the conformer concentrations on the short and long chains of these mixed-chain PCs was fatally compromised.

^{2}H-NMR spectroscopic studies of hydrocarbon chain orientational order

Illustrated in Fig. 2 are the observed and "dePaked" ^{2}H-NMR spectra of the *sn*1 and *sn*2 chain-perdeuterated analogs of 10:22 PC. As expected, only a few of the quadrupolar splittings arising from deuterons at different positions on the respective fatty acyl chains are resolved in both the normal and the dePaked spectra. However, smoothed orientational order profiles can be obtained from these data if one assumes that orientational order decreases progressively towards the methyl terminus of the fatty acyl chain and that each CD_2 group makes a comparable contribution to the observed spectral intensity (see Lafleur et al., 1989). Using such assumptions, orientational order profiles of the various lipids studied were calculated from their dePaked spectra and plotted as a function of hydrocarbon chain number (Figs. 3-5).

The orientational order parameter profiles of both the short and long hydrocarbon chains of 20:12 PC and 10:22 PC bilayers at temperatures just above their respective T_m values are presented in Fig. 3. When viewed as a function of carbon number, the orientational order parameters of the shorter of the two hydrocarbon chains of these lipids are smaller than those occurring at comparable carbon numbers of the long chains. Moreover, the shape of the order parameter profiles of the short and long hydrocarbon chains of each lipid differ significantly, regardless of whether the short or long chains are esterified at the *sn*1 or *sn*2 positions of the glycerol backbone. With both of these asymmetric-chain lipids, the short hydrocarbon chains exhibit conventional order profiles similar to those observed with typical symmetric-chain lipids. In each case, the S_{CD} values decrease slightly as a function of distance from the carboxyl group towards the middle of the chain and, beyond this so-called order plateau region, they decrease progressively more rapidly towards the methyl terminus. With the longer of their two hydrocarbon chains, the orientation order profiles also exhibit plateau regions near to their respective carboxyl groups, and beyond these regions the S_{CD} values decrease progressively with increasing distance from the carboxyl group. However, in marked contrast to shorter hydrocarbon chains of these lipids, the rate of decline of S_{CD} in their long chains is greater in the central region than it is near the methyl terminus of the chains. As illustrated in Fig. 3, the steep decline in S_{CD}, which occurs just beyond their plateau regions, slows significantly as the methyl terminus is approached, after which S_{CD} values decline markedly at the terminal-methyl group. The shapes of the orientational order profiles of the long chains of these lipids are not typical of those observed with any of the symmetric chain lipids that have been studied so far. Indeed, an examination of the order profiles exhibited by the eicosanoyl chains of 20:12 PC and by those of 20:20 PC (see below) indicates that the unusually shaped order profile of the former is attributable to the hydrocarbon chain length asymmetry of the lipids studied and not to the length of their long hydrocarbon chain per se.

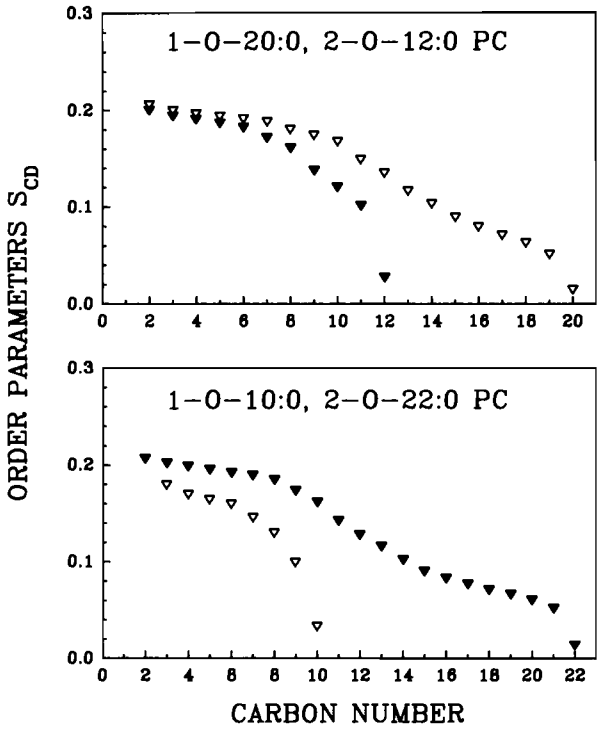

FIGURE 3 ^{2}H-NMR order profiles derived from the dePaked spectra of specifically chain-perdeuterated analogs of 10:22 PC (*bottom* panel) and 20:12 PC (*top* panel) at temperatures just above T_m. The orientational order parameters are plotted as a function of hydrocarbon chain number. The open symbols describe the order profiles obtained from *sn*1 chain-perdeuterated lipids, and the filled symbols describe the order parameters obtained from the *sn*2 chain-perdeuterated lipids.

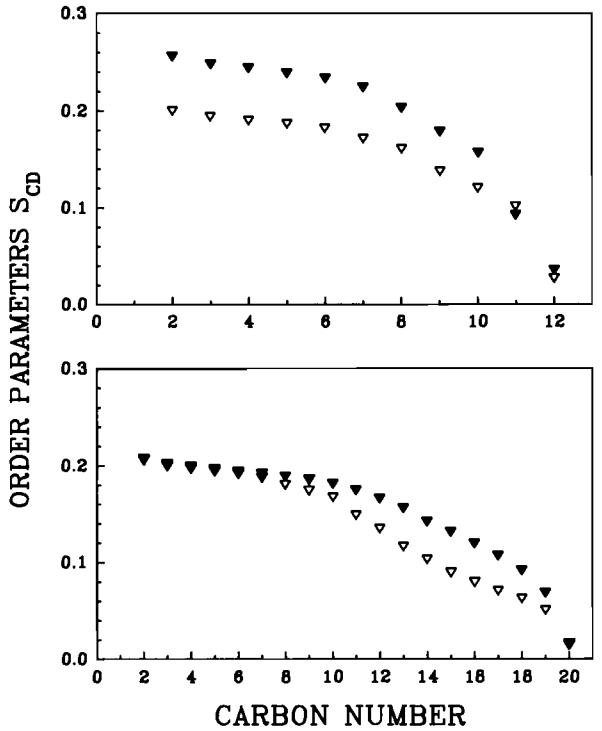

FIGURE 4 Comparison of the ^{2}H orientational order profiles of the long and short chains of the asymmetric chain PC with those of long- and short-chain symmetric chain PC. Each of the order profiles shown was derived from spectra acquired at comparable reduced temperatures (T_m + 6°C). The top panel shows the orientational order profiles described by the dodecanoyl chains of 20:12 PC (▽) and *sn*2-d_{23} 12:12 PC (▼). The bottom panel shows the orientational order profiles described by the eicosanoyl chains of 20:12 PC (▽) and chain-perdeuterated 20:20 PC (▼).

Illustrated in Fig. 4 are comparisons of the orientational order parameter profiles of the short and long hydrocarbon chains of the chain-asymmetric lipid, 20:12 PC, with those of the short and long symmetric-chain lipids 12:12 PC and 20:20 PC, respectively. The order profiles shown therein were calculated from spectra acquired at temperatures just above the T_m values of the lipids studied. As illustrated in Fig. 4 (*top*), the dodecanoyl chain exhibits qualitatively similar orientational order profiles in the chain-asymmetric and chain-symmetric PCs studied. However, at comparable reduced temperatures (T_m + 6°C), the dodecanoyl chains of the asymmetric-chain lipid 20:12 PC exhibit significantly lower chain-averaged orientational order parameters ($\langle S \rangle$ = 0.153) than do the dodecanoyl chains of the symmetric-chain species, 12:12 PC ($\langle S \rangle$ = 0.193). The difference between the order profiles of the dodecanoyl chains of the chain-symmetric and -asymmetric lipids is most pronounced in the plateau regions and are minimal near their methyl termini where the S_{CD} values tend to converge.

The bottom panel in Fig. 4 shows the orientational order profiles of the eicosanoyl chains of the chain-symmetric and -asymmetric PCs, 20:20 PC and 20:12 PC, respectively. It is evident that the shape of the order profile of the symmetric-chain species differs significantly from that of the asymmetric-chain lipid. Specifically, the order gradient described by CD_2 segments adjacent to the terminal methyl group of 20:20 PC increases as the terminal methyl group is approached, in marked contrast to the asymmetric-chain lipid, for which the orientational order gradient adjacent to the terminal methyl group is relatively shallow. This result clearly demonstrates that the difference between the shapes of the order profiles shown in the bottom panel of Fig. 4 is not a function of the length of the hydrocarbon chain per se, but is caused by the differences in the chain length asymmetries of the two lipids concerned. At temperatures just above the respective T_m values of the two lipids, the order profiles of the eicosanoyl chains in the symmetric- and asymmetric-chain PC bilayers exhibit comparable S_{CD} values in the plateau regions adjacent to their carboxyl groups. However, the plateau region of the eicosanoyl chains of 20:12 PC is shorter than that of the eicosanoyl chains of 20:20 PC and, as a result, the S_{CD} values of the two eicosanoyl chains diverge significantly in the mid-regions of their respective order profiles, although they tend to converge near their methyl termini. When examined at comparable reduced temperatures (T_m + 6°C), the chain-averaged order parameter value of the eicosanoyl chains of the symmetric chain lipid ($\langle S \rangle$ = 0.155) is larger than that of the eicosanoyl chain of the asymmetric-chain lipid 20:12 PC ($\langle S \rangle$ = 0.136). It is interesting that the $\langle S \rangle$ values of both the short and long hydrocarbon chains of the asymmetric-chain species are smaller than those of the corresponding hydrocarbon chains of the comparable symmetric-chain PCs. This observation,

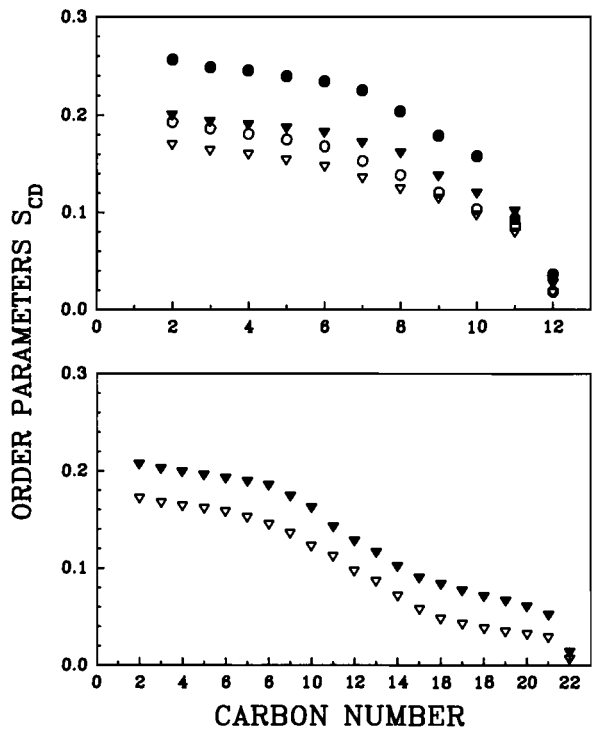

FIGURE 5 Effect of temperature on the ^2H orientational order profiles of the dodecanoyl chains of *sn*2-d_{23} 12:12 PC PC (*top*, ○, ●), the dodecanoyl chains of 20:12 PC (*top*, ▽, ▼) and the docosanoyl chains of 10:22 PC (*bottom*). The closed symbols describe order profile obtained from spectra acquired at T_m + 6°C, whereas the open symbols describe the order profiles obtained from spectra acquired at T_m + 40°C.

and the FTIR spectroscopic data presented above, indicate that whether normalized for hydrocarbon chain length or the total number of carbon atoms in the fatty acyl chains, the hydrocarbon chains of the asymmetric-chain PCs are generally more disordered than are those of the appropriate symmetric-chain counterparts. The structural significance of these data will be examined in the Discussion.

An examination of temperature-dependent changes in the orientational order profiles of these asymmetric-chain lipids reveals other noteworthy aspects of the orientational properties of their hydrocarbon chains. The top panel in Fig. 5 shows the orientational order profiles of the dodecanoyl chains of 20:12 PC and 12:12 PC at temperatures just above and well above their T_m values (i.e., at T_m + 6 and 40°C, respectively). As expected, the orientational order parameters of both lipids decrease as the temperature increases and the greatest decreases in the magnitude of S_{CD} occur in the plateau region of the order profile. At comparable reduced temperatures over the temperature range studied, the S_{CD} values of the dodecanoyl chains of the asymmetric-chain lipid (20:12 PC) are also lower than those exhibited by the dodecanoyl chains of the symmetric-chain lipid (12:12 PC). However, we find that the temperature-dependent decreases in hydrocarbon chain order are considerably greater with the symmetric-chain lipid species. Thus, when examined at the same reduced temperature, the orientational order profiles of the two lipids become more similar with increasing temperature and tend to converge. In our studies of the order profiles of the long chains of the asymmetric-chain PCs and those of the appropriately long, symmetric-chain PCs, we also find that their S_{CD} values decrease with increases in temperature above T_m, although the magnitude of the decreases appears to be smaller than observed with either the symmetric short-chain PC or the short chains of the asymmetric-chain lipid. Unfortunately, a comparable study of the effect of temperature on the order profiles of the long chains of the asymmetric-chain PC with those of the appropriate symmetric-chain counterpart was precluded by the fact that the high temperatures (≥90°C) required for studying symmetric-chain PCs such as 20:20 PC and 22:22 PC results in significant hydrolytic degradation of these lipids. With these particular symmetric-chain PCs, the observed decreases in S_{CD} in the experimentally viable temperature range ($\leq T_m$ + 15°C) were too small for us to make a reliable and conclusive comparison. In the case of the asymmetric-chain lipid, however, we did find that the increases in temperature in the range T_m to T_m + 40°C result in comparable decreases in the measured S_{CD} values throughout the entire hydrocarbon chain save the terminal methyl group (Fig. 5, *bottom*). This temperature-dependent behavior differs markedly from that of the symmetric chain lipids, for which increases in temperature result in relatively large decreases in S_{CD} values in the plateau region and smaller decreases in the S_{CD} values of CD_2 groups near the methyl terminus (see Seelig and Seelig, 1974). That such temperature-dependent decreases in S_{CD} also occur near the methyl termini of the long chains of these asymmetric-chain PCs is significant because it indicates that the unusually shallow orientational order gradient exhibited by the CD_2 groups near the methyl termini cannot be attributed to an approach to some limiting orientational order. Thus, the shallow orientational order gradient observed near the methyl termini of the long chains of the chain-asymmetric lipids indicates that there are constraints on the reorientation of the methyl terminal halves of these hydrocarbon chains that differ significantly from those operating on the hydrocarbon chains of bilayers composed of symmetric-chain lipids. This conclusion has significant structural implications, which will be explored in the Discussion.

Other interesting features of the data emerge when the orientational order profiles of the short and long chains of the two asymmetric chain lipids are compared as a function of the equivalent depth of penetration of the lipid bilayer instead of the equivalent carbon number (see Fig. 6). For the purposes of this presentation, the equivalent depth is determined relative to the position of the carbonyl carbon of the *sn*1 fatty acyl chain of the same lipid molecule (i.e., the carbonyl carbon of the *sn*1 acyl chain is assigned position 1). It is also assumed that the depth of bilayer penetration of all *sn*2 carbon atoms is effectively decreased by some two methylene segments because of conformational differences between the *sn*1 and *sn*2 fatty acyl chains. When examined in this manner, it is evident that the order parameter profiles of the two short and two long chains are very similar in both 20:12 PC and 10:22 PC bilayers (see Fig. 6). This is in marked contrast the pattern observed when the orientational order parameters of

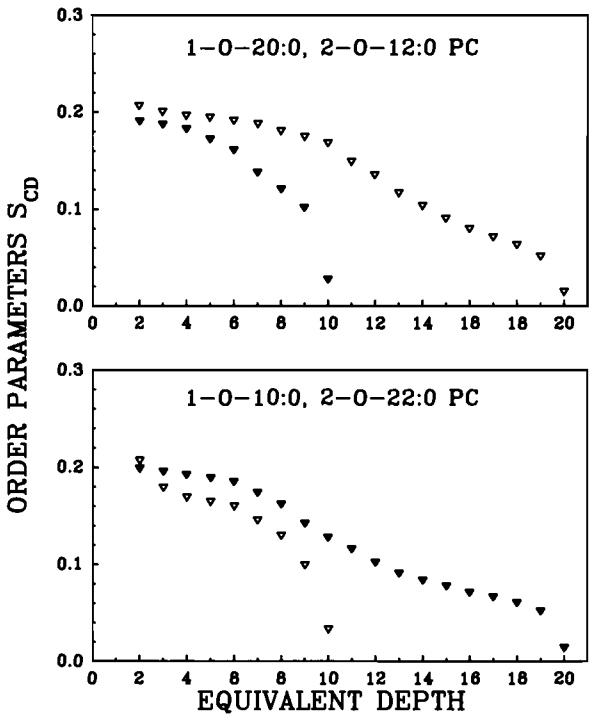

FIGURE 6 Comparison of the ^2H orientational order profiles described by the long and short chains of 20:12 PC (*top*) and 10:22 PC (*bottom*) as a function of the equivalent depth of the lipid bilayer. The open symbols describe the orientational order profiles of the *sn*1 fatty acyl chains, and the closed symbols describe the orientational order profiles of the *sn*2 fatty acyl chains. The data shown were all acquired at T_m + 6°C.

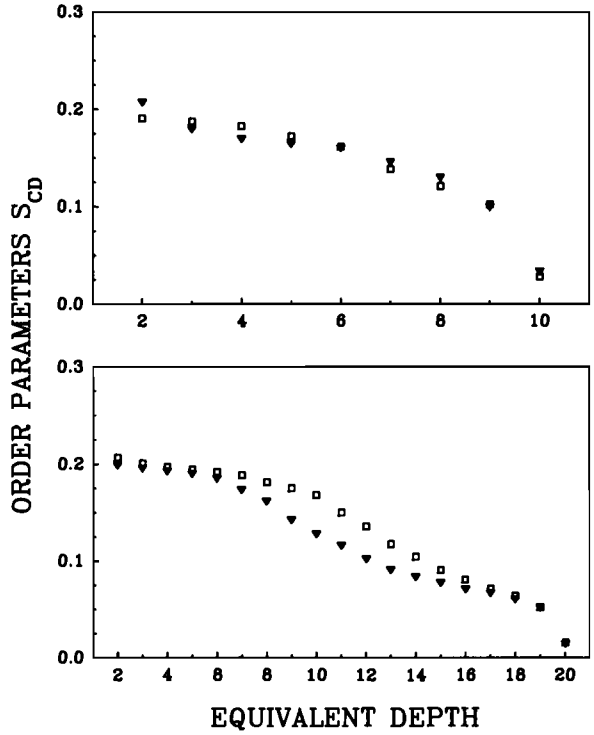

FIGURE 7 Comparison of the ^2H orientational order profiles of 20:12 PC and 10:22 PC as a function of the equivalent depth of the lipid bilayer. The orientational order profiles of the short chains (*top*) and long chains (*bottom*) are compared at T_m + 6°C. The filled symbols define the order profiles of 10:22 PC, and the open symbols define the order profiles of 20:12 PC.

the short and long chains of these lipids are plotted as a function of carbon number (see Fig. 3). As plotted in Fig. 3, the data seem to suggest that S_{CD} values in the plateau regions of the long and short chains of these mixed-chain lipids are similar when the short chains are located at the *sn*2 positions of the glycerol backbone, but differ markedly when the short chains are esterified at the *sn*1 position. The same data, when replotted with appropriate correction for the expected conformational inequivalence between the *sn*1 and *sn*2 fatty acyl chains of 1,2 diacyl PCs, clearly indicate that this is not the case. However, the remarkable feature of the "corrected order profiles" shown in Fig. 6 is that at nominally equivalent depths in the lipid bilayer, the orientational orders of the short and long chains are markedly different. Thus, at equivalent depths in the lipid bilayer, the methylene segments on the short chains of the same lipid molecule are more orientationally disordered than are comparable methylene segments on the long chains, regardless of whether the chains are located at the *sn*1 and *sn*2 positions of the glycerol backbone. This result is markedly different from that observed in comparable studies of symmetric-chain PCs, where the order parameter profiles of the two fatty acyl chains tend to be very similar in the plateau region of the order profile, although some divergence tends to occur towards the terminal methyl groups (see Seelig and Seelig, 1974).

Another noteworthy feature emerges when the order parameter profiles of the short and long chains of 10:22 PC are compared with the those of the short and long chains of 20:12 PC as a function of the equivalent depth in the lipid bilayer (see Fig. 7). These two lipids are well suited for such comparisons because the effective lengths of the short and long chains of each lipid are very close to those of the corresponding acyl chains of the other. Indeed, it has been shown that these two particular mixed-chain lipids exhibit near ideal mixing in all proportions (Xu et al., 1987). The data shown in Fig. 7 indicate that at comparable reduced temperatures above T_m, the qualitative and quantitative aspects of the orientational order profiles described by the short chains of these lipids are virtually identical. Moreover, the magnitude of the S_{CD} values of the longer chain in the plateau regions of the two lipids are very similar to each other at comparable reduced temperatures, as are the S_{CD} values of the methylene groups adjacent to the terminal methyl groups of the two lipids (see Fig. 7). However, the S_{CD} values diverge significantly in those regions of the order profile where the orientational order gradient is greatest. This is primarily because the plateau region of the orientational order profile of the eicosanoyl chain of 20:12 PC extends deeper into the bilayer than does that of the docosanoyl chain of 10:22 PC (see Fig. 7).

DISCUSSION

Several interesting conclusions can be drawn from the results of these spectroscopic studies. Our FTIR spectroscopic results suggest that there is a greater degree of hydrocarbon

chain disorder in the liquid-crystalline states of these asymmetric-chain PCs than there is in comparable symmetric-chain PCs at comparable reduced temperatures. This is reflected by a higher concentration of gauche conformers in both 20:12 PC and 10:22 PC than occurs in 16:16 PC, a symmetric-chain PC whose acyl chains contain the same number of carbon atoms. Interestingly, previously published x-ray diffraction studies of liquid-crystalline PC bilayers indicate that the bilayer thickness of 16:16 PC (see Marsh, 1990) exceeds that of 22:12 PC (see Zhu and Caffrey, 1993), despite the fact that acyl chains of the former contain fewer carbon atoms. Thus, at comparable reduced temperatures above T_m, the asymmetric-chain lipids studied here should form bilayers that are significantly thinner than those formed by 16:16 PC. Given that the hydrocarbon chains of these lipids all contain the same number of hydrocarbon atoms (i.e., the excluded volumes of their hydrocarbon chains are identical), these asymmetric-chain lipids could only form thinner liquid-crystalline bilayers than 16:16 PC if they occupy greater cross-sectional areas under such conditions. The x-ray diffraction results are thus consistent with our conclusion that the hydrocarbon chains of these highly asymmetric-chain PCs are more conformationally disordered than are those of comparable symmetric-chain PCs at comparable reduced temperatures in the liquid-crystalline state. The FTIR spectroscopic data also indicate that this is primarily attributable to an elevation in the concentration of kink and double-gauche conformers coupled with a decrease in the concentration of end-gauche conformers. Unfortunately, because of the technical difficulties described above (see Results), we were unable to estimate the distribution of these conformers between the short and long hydrocarbon chains of these lipids.

Although our FTIR spectroscopic estimates of the concentrations of nonplanar conformers in liquid-crystalline 16:16 PC bilayers are generally similar to those obtained in previous studies (see Casal and McElhaney, 1990; Senak et al., 1991), the absolute values that we obtained (particularly those of the concentration end-gauche conformers) differ somewhat from those reported previously. We believe that this discrepancy is almost entirely attributable to the different methods used to construct a reference baseline for the absorption peaks of interest. Specifically, we constructed baselines using cubic spline interpolation methods, whereas in previous studies the baseline was constructed by connecting a straight line between absorption minima near 1396 and 1320 cm^{-1}. Indeed, we find that there is closer agreement between our estimates and previously published work if our analyses were performed using baselines similar to those used previously (see Casal and McElhaney 1990; Senak et al., 1991). That the integrated areas of the absorption peaks of interest are so dependent upon the way in which the baseline is constructed is probably because these are intrinsically weak bands that, in the case of hydrated phospholipid bilayers, are located in a "valley" between two larger peaks. Thus, with phospholipid bilayers potential errors in the determination of this type of quantitative information are significantly greater than is the case with samples such as pure alkanes. Therefore, the absolute value assigned here to each conformer type should be considered an estimate only. Nevertheless, within the context of this particular study, such uncertainties are not critical because the main conclusions drawn from our results are derived from comparisons of data acquired under similar conditions and processed by the same methods. Also, the principal conclusions drawn are unaffected by the way in which our results were analyzed.

The major finding of our ^{2}H-NMR spectroscopic studies is that the orientational order profiles of the short and the long chains of the asymmetric-chain PCs studied are significantly different. Although some differences between those properties of the short and long hydrocarbon chains were expected, there are some unusual aspects of the data presented. In particular, the ^{2}H-NMR orientational order parameter profiles exhibited by the long chains of these lipids indicate that methylene segments in the methyl terminal half of the hydrocarbon chains are relatively orientationally disordered and exhibit an unusually shallow orientational order gradient compared with comparable symmetric-chain PCs. Moreover, our results clearly indicate that the unusual orientational properties of the long chains of the asymmetric-chain PCs are caused by chain length asymmetry and not the absolute length of the long hydrocarbon chains, per se. Also, the shallow order gradient described by the highly disordered CD_2 segments near the ends of the long fatty acyl chains of these molecules cannot be attributed to their approaching some limiting S_{CD} values, because large decreases in the S_{CD} values of this part of the hydrocarbon chain continue to occur when the samples are heated to temperatures well above T_m. Thus, we suggest that the reorientation of methylene segments near the ends of the long hydrocarbon chains of these asymmetric-chain lipids are subject to constraints that are different from those occurring in symmetric-chain bilayers. Further, when one considers the structure of these mixed-chain lipid molecules, it seems unlikely that such constraints could be imposed by either the short chains or the long chains of adjacent lipid molecules in the same monolayer. Therefore, we conclude that these constraints must arise because of interactions of the long hydrocarbon chains of lipids in one monolayer with the hydrocarbon chains of lipids in the opposite monolayer. Similarly shaped orientational order profiles were also observed in ^{2}H-NMR studies of labeled glycosphingolipids dispersed in 1-stearoyl, 2-oleoyl PC bilayers and were ascribed to the reorientational constraints caused by interaction of the ends of the long chains of the glycosphingolipid molecules with hydrocarbon chains in the opposing monolayer (see Morrow et al., 1992, 1993). The suggestion that there is interaction between the hydrocarbon chains of the two opposed monolayers of these particular liquid-crystalline bilayers implies that the opposing monolayers are not completely uncoupled even when the hydrocarbon

chains are melted (i.e., some form of hydrocarbon chain interdigitation persists in the liquid-crystalline phase). However, that the type of hydrocarbon chain interdigitation that is envisaged in the liquid-crystalline phases of these bilayers is of a statistical nature in which the ends of the long hydrocarbon chains of one monolayer are rapidly sampling both their own monolayer and regions of the opposing monolayer. This situation should not be confused with the static structural format that occurs in the mixed-interdigitated gel phases that these lipids form when cooled to temperatures below T_m (see Huang (1990), Lewis and McElhaney (1993), and references cited therein). The possibility that some form of transient, partial hydrocarbon chain interdigitation persists in the liquid-crystalline phase has been suggested by previous MAS-NMR and x-ray diffraction studies (see Halladay et al., 1990; Zhu and Caffrey, 1993). Our work is compatible with such a suggestion. Indeed, it is also possible that the unusual shape of the ^{2}H orientational order profiles described by us and by others (see Morrow et al., 1992, 1993) is a general "signature" of interdigitated chains in liquid-crystalline lipid bilayers.

The possibility that the two monolayers of this class of lipid bilayer are not completely uncoupled in the liquid-crystalline state is especially significant when one considers the results of a recent study by Lewis et al. (1994). These workers found that the thermotropic phase behavior observed upon cooling aqueous dispersions of these mixed-chain PCs from their respective liquid-crystalline phases is dependent upon the thermal history of the sample in the gel state. Specifically, before extensive low-temperature incubation of the gel phase, these mixed-chain lipids reproducibly exhibit single cooling exothermic phase transitions when cooled from the liquid-crystalline phase. However, after extensive low-temperature incubation of the gel phase, two cooling exothermic phase transitions are observed. Moreover, the combined enthalpy of the two cooling exotherms is the same as that of the single exotherm originally observed, and both cooling exotherms are liquid-crystalline to gel phase transitions. Currently, the molecular basis of this behavior is unknown. However, Lewis et al. (1994) suggested that the above observations could be the result of changes in the domain structure of the lipid assembly upon low-temperature incubation of the gel phase, and they further speculated that the two cooling exothermic transitions observed could be attributable to the differential freezing of differently sized lipid domains. Interestingly, although the authors did not suggest any mechanism by which the "memory" of the domain structures formed in the gel phase could be retained in the liquid-crystalline state, they did recognize that the plausibility of their suggestion (or, indeed, any other suggestion that might be offered) is dependent upon the existence of such a mechanism. Given that at low temperatures the gel phases of these lipids form compact domains in which the opposing monolayers are tightly coupled (see Lewis and McElhaney (1993) and references cited therein), our evidence that the two monolayers are not completely uncoupled when the hydrocarbon chains melt suggests a possible mechanism whereby some memory of the domain structures formed in the gel phase could be retained at liquid-crystalline temperatures.

Finally, our ^{2}H-NMR results also demonstrate that in the region near to the polar/apolar interfaces of these asymmetric-chain bilayers, methylene units located on short chains are more disordered than are those located on long chains anchored to the same monolayer. This observation can be explained by considering that the occurrence of "defect structures" likely to nucleate the formation of nonplanar conformers is greater near the methyl termini of melted hydrocarbon chains and diminishes sharply as one progresses away from the free ends of the hydrocarbon chains (see Snyder et al., 1983). With these asymmetric-chain bilayers, the region near the polar/apolar interfacial region of any given monolayer is closer to the methyl termini of the short hydrocarbon chains than to the free ends of long chains anchored to the same monolayer. Consequently, when nonplanar conformers occur near the polar/apolar interfaces of these lipid bilayers, there is a greater probability that they will be formed in the shorter of the two hydrocarbon chains. Interestingly, such considerations might also apply to short-chain amphiphilic molecules that are incorporated into longer-chain lipid bilayers and are constrained by being anchored to the bilayer surface. That our data on the short chains of these asymmetric-chain lipids can provide insight into the conformational disposition of such molecules is supported by previous studies that demonstrate that when short chain amphiphiles are incorporated into longer chain host lipid bilayers, their hydrocarbon chains are more flexible than those of the host lipid at comparable depths within the bilayer (Reborias and Marsh, 1991).

In conclusion, the structural picture that emerges from this and previously published work on this unique class of asymmetric-chain PCs is one in which their liquid-crystalline phases are thinner and more conformationally disordered than are those formed by n-saturated symmetric-chain PCs whose acyl chains contain the same number of carbon atoms. Moreover, the individual acyl chains of these lipids are more conformationally disordered than they would have been if they were located in bilayers of symmetric-chain PCs composed of the same acyl chains. Also, the short chains of these chain-asymmetric lipids are more conformationally disordered than are the long chains at comparable depths within the bilayer. Finally, the data suggest that some form of hydrocarbon chain interdigitation exists in the liquid-crystalline state. Further refinement of this picture is the focus of continuing study.

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