^{2}H Nuclear Magnetic Resonance Order Parameter Profiles Suggest a Change of Molecular Shape for Phosphatidylcholines Containing a Polyunsaturated Acyl Chain

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ABSTRACT Solid-state ^{2}H nuclear magnetic resonance spectroscopy was used to determine the orientational order parameter profiles for a series of phosphatidylcholines with perdeuterated stearic acid, 18:0_{d35}, in position sn-1 and 18:1ω9, 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, or 22:6ω3 in position sn-2. The main phase transition temperatures were derived from a first moment analysis, and order parameter profiles of sn-1 chains were calculated from dePaked nuclear magnetic resonance powder patterns. Comparison of the profiles at 37°C showed that unsaturation causes an inhomogenous disordering along the sn-1 chain. Increasing sn-2 chain unsaturation from one to six double bonds resulted in a 1.6-kHz decrease in quadrupolar splittings of the sn-1 chain in the upper half of the chain (or plateau region) and maximum splitting difference of 4.4 kHz at methylene carbon 14. The change in chain order corresponds to a decrease in the 18:0 chain length of 0.4 $\pm$ 0.2 Å with 18:2ω6 versus 18:1ω9 in position sn-2. Fatty acids containing three or more double bonds in sn-2 showed a decrease in sn-1 chain length of 0.7 $\pm$ 0.2 Å compared with 18:1ω9. The chain length of all lipids decreased with increasing temperature. Highly unsaturated phosphatidylcholines (three or more double bonds in sn-2) had shorter sn-1 chains, but the chain length was somewhat less sensitive to temperature. The profiles reveal that the sn-1 chain exhibits a selective increase in motional freedom in a region located toward the bottom half of the chain as sn-2 unsaturation is increased. This corresponds to an area increase around carbon atom number 14 that is three to four times greater than the increase for the top part of the chain. A similar asymmetric decrease in order, largest toward the methyl end of the chain, was observed when 1-palmitoyl-2-oleoyl phosphatidylethanolamine goes from a lamellar to an inverse hexagonal (H_{II}) phase. This is consistent with a change to a more wedge-shaped space available for the acyl chain.

INTRODUCTION

Although it remains unresolved exactly how unsaturation participates in membrane function or organization, it is evident that, particularly in certain neural membranes, the inclusion of double bonds is critical for proper function. For example, near-native levels of 50 mol % of the 22:6ω3 fatty acid (Miljanich et al., 1979) are required in retinal rod outer segment disk membranes for full production of metarhodopsin II, an important photoproduct of the visual receptor rhodopsin (Wiedmann et al., 1988; O'Brian et al., 1977). In synaptosomes, where 22:6ω3 is also abundant, dietary modification resulting in depletion of 22:6ω3 and its subsequent replacement with 22:5ω6 leads to functional impairment (Salem et al., 1986; Salem, 1989). Thus, it is important that the biophysical properties of unsaturated lipids be investigated to gain a better understanding of their role in membrane function and organization.

The long chain fatty acids that make up the hydrocarbon interior of membranes align with their long axes parallel to one another but maintain a significant degree of flexibility in the biologically relevant liquid crystalline state. One way to characterize the motions and the average conformation of fatty acids is to determine the ^{2}H nuclear magnetic resonance (NMR) order parameter profiles obtained from phospholipids containing perdeuterated saturated chains in position sn-1. An order parameter profile gives an impression of the flexibility gradient along the deuterated chain and/or tilt of the chain to the bilayer normal. A change in the average chain order parameter can also be interpreted as a change in length of the sn-1 chain and, if the chain volume is known, as a change in the chain cross-sectional area (Seelig and Seelig, 1974; Bloom and Mouritsen, 1988; Thurmond et al., 1991; Thurmond et al., 1993; Nagle, 1993). The shape of a lipid molecule and its area at the lipid–water interface are influenced by the presence of unsaturation, and this may affect membrane properties such as curvature and membrane protein functionality (Israelachvili et al., 1980; Deese et al., 1981; Paddy et al., 1985; Gruner, 1985; Wiedmann et al., 1988; Mitchell et al., 1992; Slater et al., 1994).

To date, biophysical investigations on unsaturation in lipid membranes have yielded no universal agreement on what function unsaturated fatty acids perform. Many characteristics have been recorded: phase transitions (Coolbear et al., 1983; Barry et al., 1991; Niebylski and Salem, 1994), quadrupolar splittings (Paddy et al., 1985; Yeagle and Frye, 1987; Rajamoorthi and Brown, 1991; McCabe et al., 1994), and bilayer thickness (Monck et al., 1992; Thurmond et al., 1994). Yet none of these properties explains fully the necessity for double bonds, especially multiple double bonds. In fact, there linger apparent contradictions between unsaturation and membrane order. We observe in this work that, at a given absolute temperature, sn-1 chains become more

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disordered as sn-2 becomes more unsaturated, yet highly unsaturated chains themselves are thought to be quite ordered structures (Dratz and Deese, 1986; Applegate and Glomset, 1986; Litman et al., 1991; Baenziger et al., 1992). In general, unsaturated membranes are more ordered than saturated membranes under conditions of equal reduced temperatures, but a greater disorder is observed in unsaturated membranes relative to saturated membranes at equal absolute temperature (Seelig and Seelig, 1977; Paddy et al., 1985; Salmon et al., 1987; Barry et al., 1991). Also somewhat puzzling is the persistant observation that when membrane proteins are included in bilayers, either reconstituted at native lipid to protein ratios or in native membranes, they have almost no effect on the overall packing of the acyl chains as measured by ^{2}H NMR (Bienvenue et al., 1982; Monck et al., 1992; Thurmond et al., 1994).

In this study, we measure the perdeuterated stearoyl ($18{:}0_{d35}$) sn-1 chain order for a series of phosphatidylcholines (PCs) containing an extended range of sn-2 unsaturated acyl chains, including 18:1ω9, 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, and 22:6ω3 to quantify the extent to which sn-1 chain order and length varies as a function of sn-2 unsaturation and chain length. The measurements were performed over a temperature range of $\pm$50°C. The order parameter profiles and the chain length were determined for the temperature range 0–50°C. What has emerged is the observation that at physiological temperature, unsaturation in sn-2 causes an inhomogeneous decrease in sn-1 chain order that is largest toward the end of the chain. This is consistent with an increase in available space for the bottom of the chain compared with the top.

MATERIALS AND METHODS

PCs containing sn-1 chain perdeuterated stearic acid ($18{:}0_{d35}$) and either 18:1ω9, 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, or 22:6ω3 in the sn-2 position were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). The extent of deuteration was measured by mass spectrometry and found to be 98% complete. We have chosen 18:0 as the sn-1 fatty acid because it is the predominant fatty acid with which long chain polyunsaturates are paired in a phospholipid molecule in neural membranes (O'Brien and Sampson, 1965; Salem et al., 1980). All of the unsaturates are naturally occurring fatty acids.

Lipid purification

Phospholipids containing fatty acids with three or more double bonds were purified by preparative high-performance liquid chromatography before NMR experiments by a method similar to that described by Holte et al. (1990). Briefly, using a reverse-phase preparative column and a 100% methanol mobile phase, the pure lipid is separated from oxidation products and other more polar degradation species. The different lipid species were detected by UV absorbance. The later eluting fraction of nonoxidized lipid was collected and the solvent removed by rotary evaporation. The antioxidant butylated hydroxy toluene (BHT) was added after purification to achieve a final lipid-to-BHT ratio of 250:1. Analytical high-performance liquid chromatography by the same method on a smaller scale was used to determine whether any significant degradation had occurred during the NMR experiments.

Sample preparation

After removal of organic solvents by rotary evaporation or under a stream of argon, the lipids were dispersed in a small volume of water and transferred to a 5-mm OD glass tube. Samples were lyophilized overnight and then rehydrated with 50 wt. % deuterium-depleted water. Centrifugation at 3000 rpm in a Beckman GS-6R centrifuge was used to concentrate the lipid at the bottom of the tube. Sample tubes were flame sealed. All manipulations were carried out under an argon atmosphere. At least two samples of each lipid species were prepared. Care was taken to ensure that samples were not orienting with the magnetic field at the higher temperatures by periodically centrifuging them back and forth in the sample tubes between measurements. Effects of magnetic orientation were judged to be negligible at temperatures of less than 50°C and a field strength of 7 T (Gawrisch, Sinnwell, and Holte, manuscript in preparation).

NMR measurements

^{2}H and ^{31}P measurements were performed on a Bruker MSL 300 spectrometer using a high-power probe with a 5-mm solenoid sample coil tunable to both ^{2}H and ^{31}P frequencies. ^{2}H NMR spectra were observed at 46.1 MHz using a quadrupolar echo pulse sequence with a 3.5-μs 90° pulse, a 30-μs delay between pulses, and a repetition rate of two acquisitions per second. A spectral width of 125 kHz was used for liquid-crystalline phase spectra, and a width of 500 kHz was used for the gel phase. The carrier frequency was placed exactly at the center of the spectrum. The free induction decay was left-shifted to ensure that the Fourier transform began exactly at the echo maximum. Proton-decoupled ^{31}P spectra, observed at 121.5 MHz, were collected with a Hahn echo sequence with a 1.75-μs 90° pulse, a between-pulse delay of 40 μs, and a repetition rate of one acquisition per second. A spectral width of 100 kHz was used.

The temperature of the gas flowing over the sample was regulated by a Bruker variable-temperature unit. Samples were equilibrated for 20–30 min in the probe after each temperature change before the measurement began. We calibrated the variable-temperature unit by placing a thermistor inside the probe coil and recording the temperature every 10 deg over the range of temperatures typically used. Temperatures are accurate within $\pm$0.5°C.

Derivation of order profiles

^{2}H NMR powder pattern spectra for the lipids in the liquid–crystalline phase were dePaked to give spectra that correspond to the 0° orientation of the lipid bilayer normal with respect to the magnetic field (Sternin et al., 1983). However, there is still considerable overlap in the dePaked spectrum, making the assignment of peaks difficult. To derive the order parameter profile for each position along the deuterated chain, we integrated dePaked spectra over the peak areas that were due to methylenes in the sn-1 chain (Lafleur et al., 1989). It is assumed that each $-CD_2$ group contributes equal intensity to the dePaked spectrum and that order is lowest at the center of the bilayer and increases for each position on the chain towards the glycerol backbone. The integral was divided into 32 steps and then averaged over 2 steps to yield 16 order parameters corresponding to each of the methylene segments in the 18:0 chain. The order parameters $S(n)$ were calculated with Eq. 1, where e^2qQ/h = 167 kHz is the quadrupolar coupling constant for ^{2}H in a C–^{2}H bond:

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2qQ}{h}S(n). \quad (1)$$

The terminal methyl splitting can be assigned unambiguously and was obtained directly from the dePaked spectrum. For the calculation of the average order parameter, a "final" methylene order parameter $S(18)$ was extrapolated from a quadratic fit of the last six points ($S(13)$–$S(17)$) of the step function (Lafleur et al., 1989). All order parameters were added together and divided by the number of deuterated carbons in the chain to yield an average order parameter $\langle S\rangle$.

Determination of phase transition temperatures

A first moment of the deuterium spectra was calculated according to the following formula:

$$M_1 = \frac{\int_0^\infty \omega f(\omega)\, d\omega}{\int_0^\infty f(\omega) d\omega}, \quad (2)$$

where $\omega = 0$ corresponds to the Larmor frequency, ω_0. The spectral first moment M_1 is known to decrease suddenly at the gel-to-fluid phase transition for single-component systems and can be used for determination of transition temperatures (Davis, 1983).

RESULTS

Effect of unsaturation on order parameter profiles

The right half of the symmetric deuterium spectra for investigated phosphatidylcholines representative of the liquid crystalline phase are shown on the left side of Fig. 1. Increasing sn-2 chain unsaturation causes a reduction of quadrupolar splittings. The reduction is larger for the methylenes near the end of the acyl chain at the bilayer center than for methylene groups near to the glycerol. As a result of this nonuniform change, the intensity of peaks near the center of the spectrum is increased. The dePaked spectra, shown on the right side of Fig. 1, were used to determine the order parameter profiles as described in the Materials and Methods section. The profiles from measurements taken at 37°C for all lipids are shown in Fig. 2. The significant changes in sn-1 chain order caused by unsaturation in sn-2 take place after the addition of two and three double bonds (versus one double bond). There is overlap at nearly every position for profiles of lipids at 37°C containing three to six double bonds. Comparison of the profiles at physiological temperature shows that an increase in the number of double bonds from 1 to 3, 4, 5, or 6 reduces the quadrupolar splittings by 1.6 kHz in the upper half of the sn-1 chain (plateau region), by 4.4 kHz in the lower third of the chain at methylene carbon 14, and by 2.6 kHz for the methylene next to the methyl. The reduction of the terminal methyl splittings was less than 1 kHz.

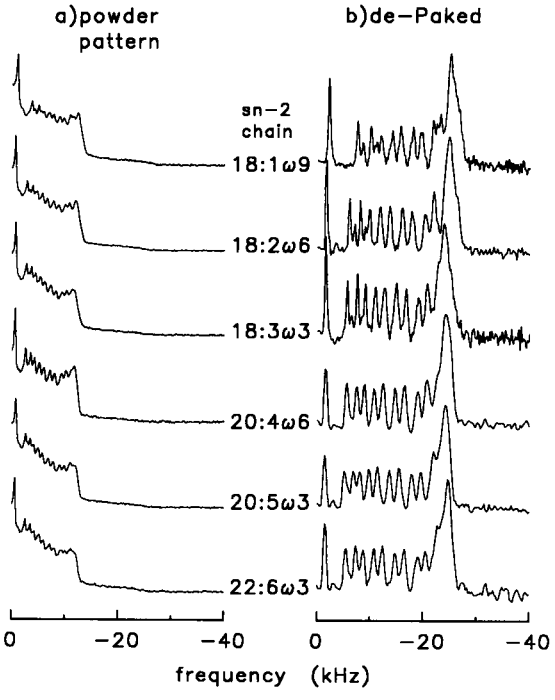

FIGURE 1 ^{2}H NMR spectra for (18:0_{d35})(sn-2)PC recorded at 37°C. The ^{2}H NMR half-spectra of the aqueous dispersion of randomly oriented bilayers are depicted at the left (*a*), and the corresponding dePaked spectra are at the right (*b*). The sn-2 chain is indicated at the center of each pair of spectra.

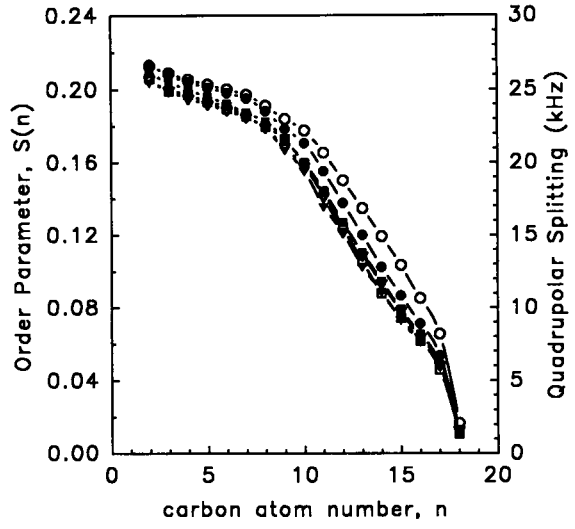

FIGURE 2 Order parameter profiles of (18:0_{d35})(sn-2) PC dispersions from spectra recorded at 37°C. The corresponding sn-2 chains are as follows: ○, 18:1ω9; ●, 18:2ω6; ▽, 18:3ω3; ▼, 20:4ω6; □, 20:5ω3; ■, 22:6ω3. The dePaked spectra of Fig. 1 (*b*) were used to calculate the order parameter profiles as a function of carbon position. Methods for deriving the order profiles are described in the text.

To understand whether the reduction in order of the sn-1 chain as a function of unsaturation is homogenous along the chain, we have calculated the difference of the order parameter profile of the 18:0_{d35} chain in position sn-1 between (18:0_{d35})(18:1ω9)PC and the other unsatured PC species at 37°C. This direct comparison of an order change, called the "difference order parameter," $\Delta S^{x,18:1}(n)$, with chain position n, was proposed by Nezil and Bloom (1992):

$$\Delta S^{x,18:1}(n) = |S^x(n)| - |S^{18:1}(n)|, \quad (3)$$

where the superscript x refers to different sn-2 chains with increasing numbers of double bonds. Double bonds in the sn-2 chain result in a reduction in order, ΔS, of the sn-1 chain that has a maximum near carbon 14, shown in Fig. 3. The reduction in order near the end of the chain is three to four times greater than at the top part of the chain near the glycerol backbone.

Calculation of the effective sn-1 chain length

Average order parameters $\langle S \rangle$ were calculated for lipids in the liquid–crystalline phase as described in the Materials and

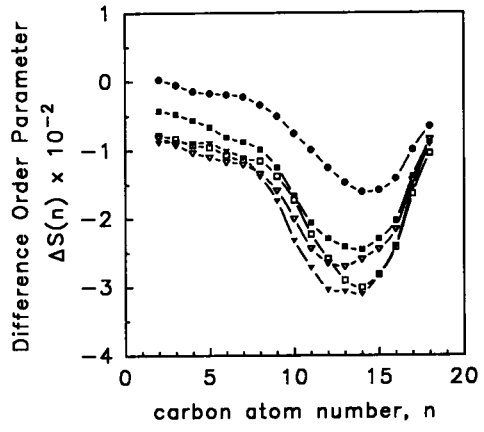

FIGURE 3 Difference order profile $\Delta S^{x,18:1}(n)$ calculated to describe the change in sn-1 chain order as a function of sn-2 unsaturation at 37°C. The order profile of the $18:0_{d35}$ in $(18:0_{d35})(18:1)$PC was subtracted from each of the other unsaturated PC species, x. Additional sn-2 double bonds result in a reduction of order in the lower half of the sn-1 chain centered around carbon position 14. The sn-2 chains (x) are denoted as follows: ●, 18:2ω6; ▽, 18:3ω3; ▼, 20:4ω6; □, 20:5ω3; ■, 22:6ω3.

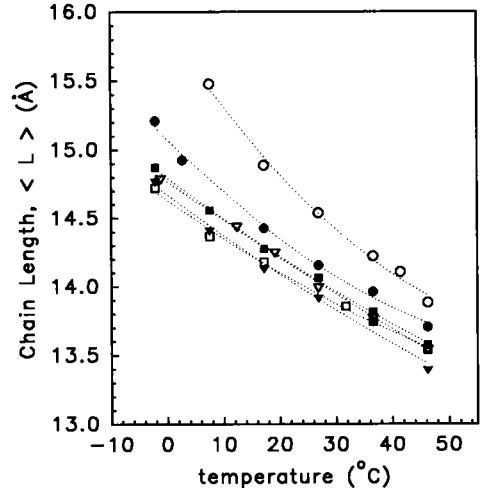

FIGURE 4 Phospholipid sn-1 chain length plotted as a function of temperature for samples with an unsaturated sn-2 chain: ○, 18:1ω9; ●, 18:2ω6; ▽, 18:3ω3; ▼, 20:4ω6; □, 20:5ω3; ■, 22:6ω3. Dashed curves represent quadratic fits to these data. Methods for deriving chain length are described in text.

Methods Section. It has been shown by a statistical model (Seelig and Seelig, 1974; Schindler and Seelig, 1975) that in the liquid–crystalline phase the average order parameter is proportional to the effective length of the saturated sn-1 chain. This lattice model has lead to the following relation (Bloom and Mouritsen, 1988; Nagle, 1993):

$$\langle L\rangle = l(0.5 + |\langle S\rangle|), \tag{4}$$

where $\langle L\rangle$ is the average projection of the sn-1 acyl chain on the bilayer normal and l is the length of an all-*trans* chain. The length l is calculated from $l = 1.27n$ Å, where n is the number of C—C bonds in the sn-1 chain and 1.27 Å is the distance between two carbon atoms projected on the long axis of the all-*trans* reference state (Bunn, 1939). The effective sn-1 chain length was calculated for lipids in the lamellar phase and is shown in Fig. 4. The acyl chain length always decreases with increasing temperature. Compared on an absolute temperature scale, increasing unsaturation of the sn-2 chain decreased the projected sn-1 chain length even though the sn-2 chain contains an additional two or four carbon atoms for PC species with more than three double bonds. The change of chain length as a function of temperature could be approximated by a quadratic equation:

$$\langle L\rangle = \langle L_0\rangle + a_1\,\Delta T + a_2(\Delta T)^2 \qquad (\Delta T = T - T_m^{warm}). \tag{5}$$

The term $\langle L_0\rangle$ corresponds to the sn-1 chain length immediately after the phase transition; a_1 and a_2 are the linear and quadratic temperature coefficients, respectively. ΔT is the difference between the actual temperature T and the chain melting temperature upon warming T_m^{warm}. The parameters $\langle L_0\rangle$, a_1, and a_2 are determined on a temperature scale that corrects for the individual phase transition temperatures of the lipids. The fit parameters are given in Table 1.

At the phase transition, PC species with 18:1ω9 and 18:2ω6 chains in sn-2 have somewhat longer sn-1 chains than the saturated di(18:0)PC and polyunsaturated species with three or more double bonds. The slope (a_1) of the chain length versus temperature curve is shallower for phosphatidylcholines with a higher degree of unsaturation, indicating that polyunsaturation lowers the sensitivity of the chain length to temperature.

Does order decrease homogeneously along the hydrocarbon chains with increasing temperature? To find out, we determined the contribution of every methylene group to the change in chain length. According to Eq. 4 the change in chain length as a function of temperature, $\Delta\langle L\rangle/\Delta T$, is related to the change of the average order parameter by the following equation:

$$\frac{\Delta\langle L\rangle}{\Delta T} = l\,\frac{\Delta\langle S\rangle}{\Delta T}. \tag{6}$$

The change of the average order parameter $\Delta\langle S\rangle$ is the weighted sum of changes of order parameters of individual methylene groups:

$$\frac{\Delta\langle S\rangle}{\Delta T} = \frac{1}{N-1}\sum_{n=2}^{N}\frac{\Delta S(n)}{\Delta T}. \tag{7}$$

N is the total number of carbon atoms in the chain, and n is the individual carbon atom number ($n = 1$ corresponds to the carbonyl group). We obtained the temperature-induced change in order, $\Delta S(n)$, by taking the difference between the order parameter profiles for every lipid at 17°C and 37°C. Plots of the function $\Delta S(n)$ are shown in Fig. 5. The plateau region, carbons 2–8, undergoes a collective decrease that is slightly smaller than that of the center region, while the sensitivity of order to temperature rapidly decreases toward the end of the chain. The results indicate that the reduction in chain length with increasing temperature can be attributed mainly to a change in order of the first two thirds of the chain

TABLE 1 Main phase transition temperatures and sn-1 chain lengths for mixed acid phosphatidylcholines

sn-2 Chain	T_m^{warm} (°C)	T_m^{cool} (°C)	$\langle L_0 \rangle$* (Å)	a_1 (Å/°C)	a_2 (Å/°C²)	sn-2 Chain Volume‡ (Å³)
18:0	52.9§	52.9	14.85	−0.049	3.4×10^{-4}	486
18:1ω9	4.8	4.8	15.59	−0.058	4.2×10^{-4}	473
18:2ω6	−12.6	−17.8	15.65	−0.049	2.7×10^{-4}	460
18:3ω3	−10.8	−13.0	15.05	−0.027	2.3×10^{-5}	447
20:4ω6	−13.5	−13.5	15.11	−0.034	9.8×10^{-5}	488
20:5ω3	−12.6	−12.6	15.02	−0.032	1.3×10^{-4}	475
22:6ω3	−2.0	−13.1	14.85	−0.030	8.8×10^{-5}	516
Average errors	±0.5	±0.5	$\pm 8 \times 10^{-2}$	$\pm 6 \times 10^{-3}$	$\pm 1 \times 10^{-4}$	–

*The data given are valid over the temperature range 50–75°C for ($18:0_{d35}$)(18:0)PC and 0–50°C for the rest of the data.
‡The chain volume was calculated according to $V_{chain} = nV_{CH} + n'V_{CH2} + V_{CH3}$ (see the Discussion subsection Area per molecule).
§Phase transitions for ($18:0_{d35}$)(18:0)PC determined by differential scanning calorimetry.

starting at the carbonyl group. This differs from the effect of sn-2 chain unsaturation, which results in a reduction of sn-1 chain length caused mainly by a decrease in order for the lower half of the hydrocarbon chain.

Main phase transitions

To determine the gel-to-liquid-crystalline phase transition temperatures, ^{2}H NMR measurements were taken over the temperature range of ±50°C every 5°C, except in the region of the phase transition where they were taken every 2°C. The first moment, M_1, was calculated from the powder spectrum and plotted as a function of temperature. The value reported as the melting temperature, T_m, was determined from the half-height of the portion of the curve where M_1 changes rapidly. The results are compiled in Table 1 for both warming and cooling runs. First, unsaturation of the sn-2 chain of phosphatidylcholines lowers the phase transition temperature by ~50°C, independently of the number and position of the double bonds. However, the reduction is not continuous,

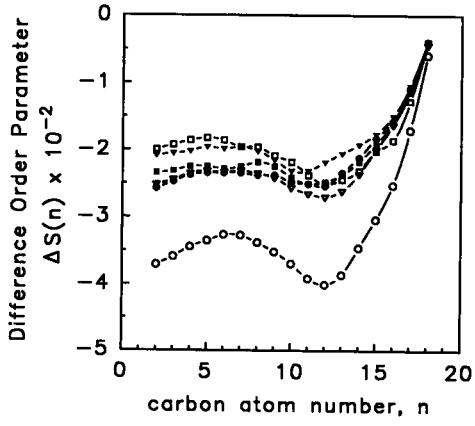

FIGURE 5 Difference order profile $\Delta S^{T2,T1}(n)$ obtained by taking the difference between the order parameter profiles for every lipid at $T2$ = 37°C and $T1$ = 17°C. The reduction in chain length with increasing temperature can be attributed to a change in order of the first two-thirds of the chain. The sn-2 chains are denoted as follows: ○, 18:1ω9; ●, 18:2ω6; ▽, 18:3ω3; ▼, 20:4ω6; □, 20:5ω3; ■, 22:6ω3.

and a minimum for the phase transition temperature has been reached when there are two double bonds in sn-2 as in the case of ($18:0_{d35}$)(18:2ω6) PCs. Increasing the number of double bonds beyond two tends to raise the transition temperature slightly and, in the case of the hexaene, 22:6ω3, the main phase transition temperature has increased by almost 10°C (for warming runs). Hysteresis was observed in species containing an 18:2ω6, 18:3ω3, or 22:6ω3 sn-2 fatty acid. The main phase transition temperatures found in this NMR study are close to values obtained by differential scanning colorimetry from the protonated species of the same lipids (Niebylski and Salem, 1994). Minor differences may be attributed to a general decrease in phase transition temperatures for lipids with deuterated chains. Further, phase transition temperatures may be sensitive to rates of heating and cooling as well as to traces of impurities, and acyl chain migration.

^{31}P NMR

Head group conformation and mobility as assessed by ^{31}P NMR were identical for all six lipids in the liquid crystalline phase. The lipids displayed lamellar spectra over the entire temperature range of investigation with a chemical shift anisotropy of −44 ± 1 ppm at 37°C. The chemical shift anisotropy decreased to −50 ppm and lower when the lipid was in the gel state, where the chains are packed in a crystalline lattice but the headgroups maintain a high degree of mobility. PCs with 18:2ω6 or 22:6ω3 in sn-2 showed the appearance of a ^{31}P NMR tensor of chemical shift without axial symmetry below temperatures of −33°C and −28°C, respectively, which is characteristic for a phase with crystalline packing of lipid head groups.

DISCUSSION

The order parameters of the sn-1 hydrocarbon chains of the investigated PC molecular species depend on unsaturation of the sn-2 chain and on temperature. From the average order parameters for the liquid-crystalline lamellar phase, a chain length projected on the normal of the lipid bilayer was cal-

culated. The measurements indicate that, at temperatures immediately above the phase transition into the liquid-crystalline state, PCs with an 18:0 or a 22:6ω3 chain in sn-2 have the shortest sn-1 chains and PCs with an 18:1ω9 or an 18:2ω6 chain in sn-2 have the longest sn-1 chain (see $\langle L_0 \rangle$ in Table 1). All lipids exhibit a reduction of sn-1 chain length with increasing temperature. Our data show that the relationship of chain length to temperature is nonlinear (see Fig. 4 and values in Table 1). The decrease in sn-1 chain length with increasing temperature is greatest at and immediately following the phase transition into the liquid-crystalline state. The rate of change declines steadily with increasing temperature. Over the investigated temperature range, the experimental data can be well approximated with a quadratic function. The linear part of the temperature coefficient (see a_1 in Table 1) decreases with increasing unsaturation. A nearly twofold difference in the coefficient a_1 exists for PCs with 18:1ω9 versus 22:6ω3 in position sn-2. There are two reasons for the lower sensitivity of chain length of polyunsaturated PC species to temperature in the physiological temperature range. First, the presence of double bonds reduces the change of chain length with temperature over the entire temperature range. Second, the chain length is less sensitive to temperature with increasing distance from the phase transition temperature into the liquid-crystalline phase. Unsaturated lipids have phase transition temperatures far below physiological temperatures, and therefore their chain lengths are even less sensitive to a temperature change at physiological temperatures.

At 37°C, PC species with increasing sn-2 chain unsaturation have more disordered sn-1 chains and therefore shorter sn-1 chains that occupy a larger cross-sectional area. We see a significant decrease of sn-1 chain order when going from 18:1ω9 to 18:2ω6, and to 18:3ω3 in sn-2. The order parameter profiles of lipids containing three to six double bonds in sn-2 overlap at nearly every chain position, indicating that the sn-1 chains have approximately the same average order and length.

Difference order parameter profiles illustrate which particular chain units have been perturbed by either a higher temperature or additional unsaturation, information concealed in an average order parameter. The change in order induced by raising the temperature results in a collective decrease in order along the first two-thirds of the chain (Fig. 5), in contrast with the change in order induced by double bonds that affects primarily the bottom half of the chain (Fig. 3). This suggests a selective disordering induced by double bonds that cannot be accomplished simply by raising or lowering the temperature.

Shape change of the sn-1 chain

A smaller order parameter of methylene groups is related to a larger deviation of the hydrocarbon chain axis from the bilayer normal, as a result of either a permanent chain tilt or rapid chain motions with components that tilt the chain away from the bilayer normal. It can be shown that both tilt and rapid chain motions reduce the length of the hydrocarbon chain projected on the bilayer normal. If the density of chain packing is constant, this results in an increase in the area per molecule. Comparison of order parameter profiles indicates that the change of order, ΔS, after the introduction of two to six double bonds into the sn-2 chain, is greatest in the bottom half of the sn-1 chain in the range of carbons atoms 9 to 16 with a maximum near carbon atom 14. Consequently, we suggest that the introduction of double bonds into the sn-2 chain results in an increase of the area of the sn-1 chain. This area increase is larger toward the methyl end of the hydrocarbon chains than near the lipid–water interface.

Our interpretation of unsaturation-induced changes in the order parameter profile of sn-1 chains is supported by changes in the smoothed order parameter profiles of 1-palmitoyl-2-oleoyl phosphatidylethanolamine molecules during a transition from a lamellar to an inverse hexagonal (H_{II}) phase (Sternin et al., 1988; Lafleur et al., 1990a, 1990b). In the H_{II} phase lipids are packed with their head groups oriented toward the center of the water-filled core. Because of the small radius of curvature of these micelles, the area available for head groups and the upper part of the chains is significantly smaller than for the end of the chain. The differences in intrachain area have been estimated for the H_{II} phase of dioleoyl phosphatidylethanolamine. In excess water at ambient temperatures the upper parts of the hydrocarbon chains occupy an area of 65 Å^2 and the ends of the chains an area of 96 Å^2 (Rand and Fuller, 1994). Therefore, in an H_{II} phase the ends of the chains occupy an area that is approximately 50% larger than for the upper parts of the chains, resulting in order along the hydrocarbon chains that decays more rapidly toward the end of the chain than for the lamellar phase. The changes in chain order with the introduction of double bonds resemble the changes observed after a phase transition to a hexagonal phase, qualitatively supporting our view that double bonds in sn-2 change the shape of the sn-1 chain to a more wedgelike average structure with a larger area per molecule toward the end of the chain.

Area per molecule

The chains in sn-2 differ not only in their chain length but also in their volume. Chain volumes can be estimated by summing a liquid-crystalline volume for each carbon unit in a chain, where $V_{chain} = nV_{CH} + n'V_{CH2} + V_{CH3}$ (V_{CH} = 20.5 Å^3, V_{CH2} = 27.0 Å^3, and V_{CH3} = 54.0 Å^3 (Marsh, 1992)). According to this calculation the chain volume of 22:6ω3 is 15% larger than the chain volume of 18:3ω3. The membrane may accommodate this extra volume by either increasing the bilayer thickness or increasing the area per lipid molecule. The experimental results obtained on the deuterated sn-1 chain suggest that the bilayer thickness does not change after more than three double bonds are introduced. Therefore, we anticipate a steady increase in the hydrophobic area per molecule up to a PC molecule with a 22:6ω3 chain in sn-2. The areas per molecule may increase by several square angstroms.

What are the consequences of area differences along the sn-1 chain for the shape of the entire lipid molecule? One possibility is that the sn-2 chain fills in any "extra space" not occupied by the sn-1 chain. If the sn-2 chain does not fill in the extra space at the interface left by the narrower upper portion of the sn-1 chain, then what we will refer to as "free volume" exists between the upper parts of neighboring molecules. Fig. 6 shows a graphic presentation of lipid molecules when they contain an sn-2 chain with one double bond, Fig. 6 *A*, or an sn-2 polyunsaturated chain (three or more double bonds, Fig. 6 *B*. We suggest that double bonds cause the acyl chains to occupy a slightly wedge-shaped space that loosens the packing at the interface but is not so pronounced as to cause formation of nonlamellar phases. Consequently, a free volume exists at the lipid–water interface between neighboring molecules that is greatest when sn-2 is polyunsaturated. We are interested in, but currently uncertain about, a possible correlation between this free volume and the fractional volume available for reorientation in the bilayer of a fluorescence probe molecule f_v, defined in the work of Straume and Litman (1987).

A bilayer having increased free volume near the upper parts of the chains close to the lipid–water interface would be more permeable for small molecules such as water. Indeed, there is evidence that small molecule permeation of the bilayer interface increases if double bonds are present (Demel et al., 1972; VanDeenen et al., 1972; Fettiplace and Haydon, 1980). The difference in molecular shape between monounsaturated and polyunsaturated lipids that we suggest may be related to the sensitivity of structural transitions of membrane receptors such as rhodopsin to lipid unsaturation. Further, proteins may more easily penetrate bilayers that are less constrained at the interface. This may explain the recently observed correlation between the activity of the membrane-bound enzymes PKC and chain unsaturation (Slater et al., 1994). It is likely that the environment offered by membranes containing polyunsaturated fatty acids is uniquely appealing to some proteins.

In conclusion, we propose that polyunsaturation causes saturated chains to become "fatter" at the bilayer center than near the interface, leading to looser packing toward the lipid–water interface. The bilayer thickness changes as a consequence of this, but only slightly. Thus, what appears to be a rather small change in thickness (vertical organization) may actually be a significant perturbation near the interface (lateral organization). It seems likely that the key to understanding unsaturation in membranes may be found in the membrane lateral organization and interfacial properties such as lipid packing, molecular cross-sectional area, the tendency for curvature, and membrane permeability.

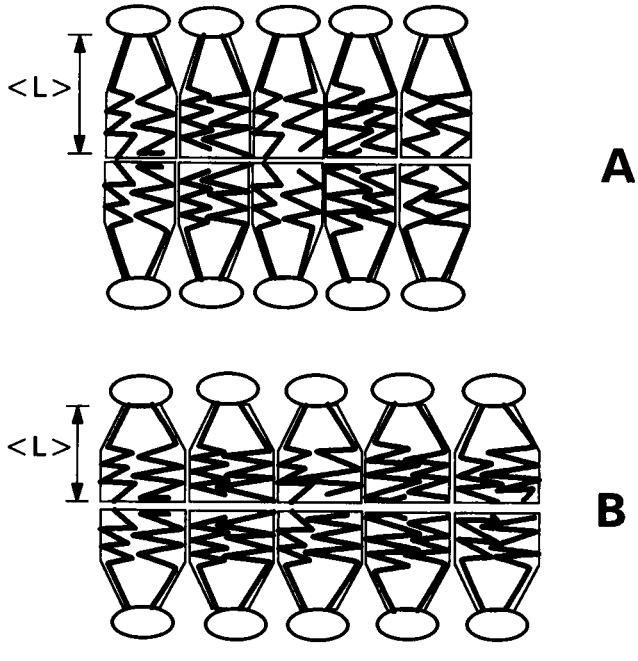

FIGURE 6 Schematic representation of molecular packing in a PC bilayer containing an sn-1-saturated chain paired with an sn-2-unsaturate containing one double bond, *A*, and three or more double bonds, *B*.

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