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Acyl chain conformational ordering in liquid-crystalline bilayers: comparative FT-IR and ^{2}H-NMR studies of phospholipids differing in headgroup structure and chain length

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Abstract. FT-IR spectroscopy has been used to evaluate the acyl chain conformational ordering of DMPC, DMPE, DMPA (pH 6 and 12), DMPG (pH 1 and 7), and DPPC, DPPE, DPPA (pH 6). The frequencies of the symmetric and antisymmetric methylene stretching vibrations were determined as a function of temperature. In the liquid-crystalline phase the frequencies show a qualitative dependence on the amount of chain disorder. Quantitative data for trans-gauche isomerization were obtained from the integral intensities of the conformation sensitive methylene wagging absorptions at ca. 1368 cm^{-1} (gtg′ and gtg sequences), 1356 cm^{-1} (double gauche) and 1342 cm^{-1} (end gauche). The integral band intensities were converted to the number of gauche conformers per acyl chain using the calibration factors published by Senak et al. (1991). At 69 °C the highest number of gauche conformers excluding contributions from single gauche conformers and jogs (g′ttg) are found for PCs (DMPC: 2.6; DPPC: 2.4), followed by $DMPG^-$ (2.0), phosphatidylethanolamines (DMPE: 1.4; DPPE: 2.0), protonated DMPG (1.5), and phosphatidic acids ($DPPA^-$: 1.7; $DMPA^-$: 1.4, $DMPA^{2-}$: 1.7). From ^{2}H-NMR measurements of perdeuterated samples of DMPC, DMPA, DPPC, and DPPA the quadrupolar splittings $\Delta\nu_{Q\perp i}$ and the order parameter S_{CDi} of the CD_2-segments close to the chain ends could be determined whereas splittings in the plateau region of the chains could not be resolved. The quadrupolar splittings are affected by trans-gauche isomerization, long axis rotation, and restricted wobbling motions of the acyl chains. In the simplest assumption, the order parameter S_{CD} can be expressed as a product of a segmental order parameter S_γ and a chain order parameter S_α. For comparison of the different lipids we used average order parameters $\bar{S}_{CD}$, obtained by averaging over all S_{CDi}-values, and $\bar{S}_\gamma$ determined from the total number of gauche conformers per chain by FT-IR-spectroscopy, to calculate an empirical average chain order parameter $\bar{S}_{\alpha\cdot}$. The combination of ^{2}H-NMR and FT-IR results allows the estimation of the relative extent of chain wobbling for the different lipid molecules. $\bar{S}_\alpha$ is lowest for PCs ($\bar{S}_\alpha \approx 0.475$) while PEs ($\bar{S}_\alpha \approx 0.51$) and PAs ($\bar{S}_\alpha \approx 0.52$) show less chain wobbling.

Key words: Phospholipids – FT-IR spectroscopy – ^{2}H-NMR spectroscopy – Chain conformations – Wobbling motions – Order parameters

Introduction

The phase behavior of model membranes is determined by the polar interactions in the head group region as well as by the hydrophobic interactions between the apolar fatty acid chains of their constituent phospholipid molecules. The transition from the gel phase to the liquid crystalline phase is accompanied by an increase in conformational disorder of the chains, i.e. an increase in the extent of trans-gauche isomerization, and the possibility for additional wobbling motion (for reviews see Cevc and Marsh 1987; Cevc 1993). Biological membranes contain a whole variety of different lipids. While it is known that some membrane proteins require specific lipids for their function the general question about the importance of the different membrane lipids is unclear. The concept of formation of lipid microdomains of different composition within the membrane is still speculative and not proven (for a discussion see Gennis 1989; Glaser 1993). As lateral diffusion is fast in the liquid-crystalline phase these microdomains dissociate and form continuously making their detection

Abbreviations: FT-IR, Fourier transform infrared, ^{2}H-NMR, deuterium nuclear magnetic resonance; DMPC(-d_{54}), (perdeuterated) dimyristoyl-phosphatidylcholine; DMPE(-d_{54}), (perdeuterated) dimyristoyl-phosphatidylethanolamine; DMPA(-d_{54}), (perdeuterated) dimyristoyl-phosphatidic acid; DMPG, dimyristoyl-phosphatidylglycerol; DPPC(-d_{62}), (perdeuterated) dipalmitoyl-phosphatidylcholine; DPPE(-d_{62}), (perdeuterated) dipalmitoyl-phosphatidylethanolamine; DPPA(-d_{62}), (perdeuterated) dipalmitoyl-phosphatidic acid; gtg, gauche$^\pm$-trans-gauche$^+$; gtg′, gauche$^\pm$-trans-gauche$^\mp$; dg, double gauche; eg, end gauche

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very difficult. The average lipid composition of these microdomains should be different as the reason for their existence is non-ideal mixing of the membrane constituents. The different domains could thus provide incorporated proteins with environments of different "fluidity". For that reason it is important to understand the conformational state of lipids in the liquid-crystalline phase and to investigate whether different types of lipids show different conformational as well as dynamical properties.

Deuterium nuclear magnetic resonance (^{2}H-NMR) and Fourier transform infrared (FT-IR) spectroscopy are complementary techniques to determine quantitatively the conformational states of the lipid acyl chains in model membranes. Both methods have the advantage of not perturbing the system under observation in contrast to, for instance, fluorescence or spin label techniques which require the introduction of a reporter group. In the past the frequencies of the symmetric and antisymmetric CH_2-stretching vibrations have been used to monitor the phase behavior of lipids as a function of temperature (Asher and Levin 1977; Amey and Chapman 1984). The phase transition from the gel to the liquid-crystalline phase is accompanied by an increase in frequency of these bands. However, despite the fact that different lipids show slightly different frequencies for these stretching bands in the liquid-crystalline phase a clear-cut quantitative relation between frequency and conformational state does not exist. This is immediately obvious when the CD_2-stretching vibrations of specifically deuterated phospholipids in the liquid-crystalline phase are measured as a function of chain position. The frequencies do not coincide with the dependence of the deuterium order parameters on segment position as observed by ^{2}H-NMR spectroscopy (Seelig 1977; Cameron et al. 1981; Hübner and Blume 1987; Blume et al. 1988).

Recently, a different approach has been used. The overall numbers of non-planar conformer sequences in the fatty acyl chains of lipids have been determined by FT-IR for a series of saturated PCs as well as some PEs by evaluation of the methylene wagging modes in the spectral region between 1395 and 1330 cm^{-1} (Casal and McElhaney 1990; Seenak et al. 1991). A related but more specific approach was used by Mendelsohn et al. (1989, 1991) and Davies et al. (1990a, b) by analyzing the conformation dependent CD_2 rocking modes of specifically deuterated PCs. Both methods trace back to calculations and experimental studies on polymethylene chains by Snyder (1967); Snyder and Poore (1973); Snyder et al. (1983) and Maroncelli et al. (1982, 1985).

While the FT-IR spectroscopic data reflect trans-gauche isomerizations in the acyl chains in the liquid-crystalline phase and report the fraction of different chain conformers, the ^{2}H-NMR quadrupolar splittings and order parameters S_{CD} are time averaged quantities. They depend not only on trans-gauche isomerization but also on long axis rotation and restricted wobbling motions of the molecular long axis (Seelig 1977; Davis 1983; Blume 1988; Davies et al. 1992). The combination of ^{2}H-NMR with FT-IR spectroscopic results promises a better understanding of the interplay between structure and dynamics in liquid-crystalline model membranes, and allows a separation of chain isomerization and long axis reorientational motions. In a previous paper we have used phospholipids deuterated at the 4-position to compare the deuterium order parameters with the FT-IR data on specific conformers. The results showed that not only differences exist between PCs and PEs, but also that the type of conformers was drastically different (Davies et al. 1992).

Here we report on the integration of FT-IR and ^{2}H-NMR results using perdeuterated phospholipids for the ^{2}H-NMR spectroscopy in order to evaluate the differences of the extent of trans-gauche isomerizations and wobbling motions of PCs, PEs and PAs and PGs. This approach uses averaged segmental order parameters determined from the wagging bands in the IR spectra and averaged deuterium order parameters and is thus not as specific and informative as the analysis of specifically deuterated lipids. Nevertheless, qualitative differences between the behavior of different phospholipid classes show up and the results can be used as a basis for further more detailed investigations.

Materials and methods

Materials

Unlabeled PCs and PEs were purchased from the following companies: Lipoid KG (Ludwigshafen, Germany), Nattermann GmbH (Cologne, Germany), Medmark GmbH (Munich, Germany) and Fluka (Neu-Ulm, Germany). Perdeuterated dimyristoyl-phosphatidylcholine, DMPC-d_{54}, and dipalmitoyl-phosphatidylcholine, DPPC-d_{62}, were synthesized using a procedure similar to that of Gupta et al. (1977). The perdeuterated fatty acids were synthesized according to a method of Dinh-Nguyen et al. (1972) by H-D exchange reactions with D_2O in the presence of a Pt-catalyst in a small autoclave. PAs were prepared from the respective PCs by transphosphatidylation with phospholipase D (Eibl and Kovatchev 1981) extracted from savoy cabbage. Deuterium depleted water was purchased from Campro Scientific (Emmerich, Germany). DSC and TLC were routinely performed to check sample integrity.

FT-IR spectroscopy

(A) Sample preparation. Multilamellar lipid dispersions (typically 10–20 wt% of lipid) were prepared by adding 50–200 µl of H_2O to 5–40 mg of dry lipid. The samples were heated to temperatures above the phase transition temperature of the respective lipid, sonicated for 15–30 s at low power using a Bandelin sonotrode (Berlin, Germany) with a 4.5 mm titanium tip, and then incubated at temperatures above the phase transition temperature of the lipid for 30 min (PCs, PGs, and PAs) or 60 min (PEs). The pH of the samples was checked and adjusted to the desired value.

(B) Instrumentation. Lipid dispersions were placed in 25 or 50 µm thick infrared cells with AgCl or BaF_2 windows. The hollow window mounts were thermostated by an external thermostat (Haake F3C, Karlsruhe, Germany). Tem-

perature control was achieved by use of a digital thermometer interfaced to a computer using a Pt 100 resistor attached close to the cell windows. Spectra were recorded with a Bruker IFS 48 Fourier transform infrared spectrometer (Karlsruhe, Germany) equipped with a MCT detector and controlled by a PC using the Spectrafile IR software (LabControl, Cologne, Germany). 512 interferograms were collected, apodized with a triangular function and Fourier transformed after one level of zero filling. Spectral resolution was 2 cm^{-1}.

(C) Data analysis. After subtraction of spectra of H_2O recorded at the same temperature from the single beam spectra of lipid samples the baseline in the 1395–1330 cm^{-1} region was still not completely flat. Therefore a linear baseline with constant slope was subtracted in the spectral region of interest (1395–1330 cm^{-1}). After spline interpolation with smoothing the resulting spectra were fitted with Gaussian-Lorentzian functions using a commercially available computer program (MINSQ II, MicroMath, Inc., Salt Lake City, UT; USA) as well as software developed in this laboratory. Both programs use the Levenberg-Marquart algorithm for non-linear least square fitting. Band intensities, bandwidths and Gaussian to Lorentzian ratio of the individual bands were allowed to be iterated freely. The initial band positions were 1378 cm^{-1} (symmetric methyl deformation mode), 1368 cm^{-1} (gtg′ and gtg sequences), 1356 cm^{-1} (double gauche sequences) and 1342 cm^{-1} (end gauche conformers). Variation of the band position was restricted to these wavenumbers ±2 cm^{-1}. The integral band intensities were normalized with respect to the symmetric methyl deformation mode and the number of specific gauche sequences was calculated using conversion factors determined from the data published by Senak et al. (1991). This procedure was applied to the spectra of at least three independent preparations of the same phospholipid.

^{2}H-NMR spectroscopy

(A) Sample preparation. Multilamellar vesicles were prepared by dispersing about 20–30 mg perdeuterated lipid in 25 µl of deuterium depleted water 10 degrees above the corresponding phase transition temperature of the lipid. If necessary, the pH of the phosphatidic acid samples was adjusted to 6–7 with small amounts of dilute HCl.

(B) Instrumentation. NMR spectra were recorded on a Bruker AMX 400 spectrometer (Karlsruhe, Germany) operating at 61.425 MHz with a fixed frequency ^{2}H-NMR probe and an additional high-power radio frequency amplifier, both from Doty Scientific, Inc. (Columbia, SC, USA). Sample temperature was controlled by a Bruker variable temperature unit. The quadrupole echo sequence was used with a 90° pulse width of 2.5 µs, a pulse spacing of 45 µs, a relaxation delay of 150 ms and a spectral width of 166.67 kHz. Typically between 4000 and 5000 FIDs were accumulated. After exponential multiplication with a line broadening of 200 Hz the FIDs were Fourier transformed using both quadrature channels, taking care to initiate the transform from the top of the echo. Spectra were recorded in order of increasing temperature and the samples were allowed to equilibrate at each temperature for ca. 60 min prior to data acquisition.

(C) Data analysis. Analysis of the quadrupole splittings was carried out with a self-written program and the WINNMR 3.0 software (Bruker-Franzen Analytik GmbH, Bremen, Germany). For "de-Pake-ing" the FORTRAN program, developed and kindly provided by E. Sternin and M. Bloom (Sternin et al. 1982), was adapted to MS-DOS-FORTRAN.

Results

FT-IR spectroscopy

The temperature dependence of the antisymmetric CH_2-stretching vibrational bands of four dimyristoyl lipids with different head groups are shown in Fig. 1 A. The phase transition is accompanied by the well-known increase

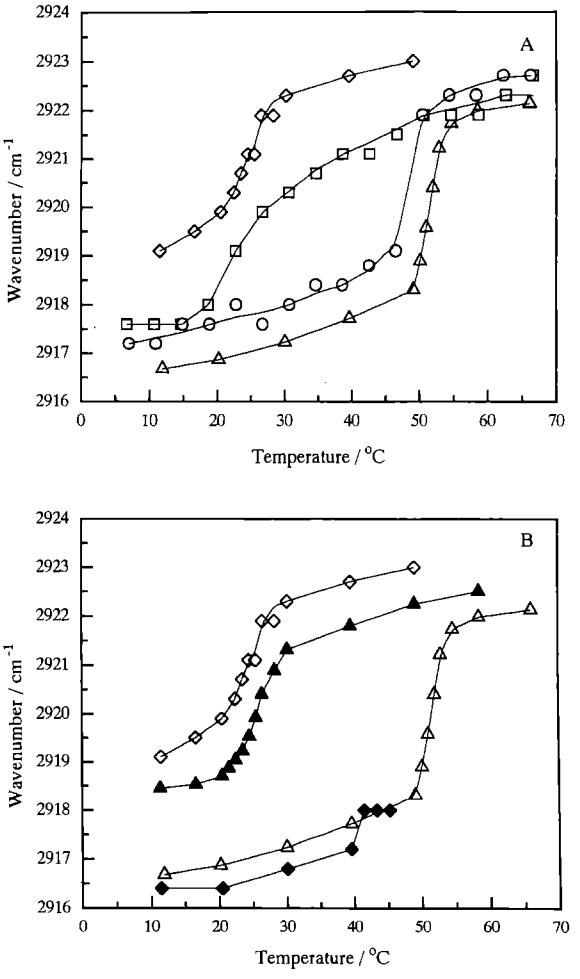

Fig. 1. A Temperature dependence of the antisymmetric CH_2 stretching vibration for DMPC (□), DMPE (○), $DMPA^-$ (△), pH 7, and $DMPG^-$ (◇) pH 8.5. **B** Temperature dependence of the antisymmetric CH_2 stretching vibration of DMPA at pH 7 ($DMPA^-$: △) and pH 12 ($DMPA^{2-}$: ▲) and DMPG at pH 8.5 ($DMPG^-$: ◇) and pH 2 (DMPG: ◆)

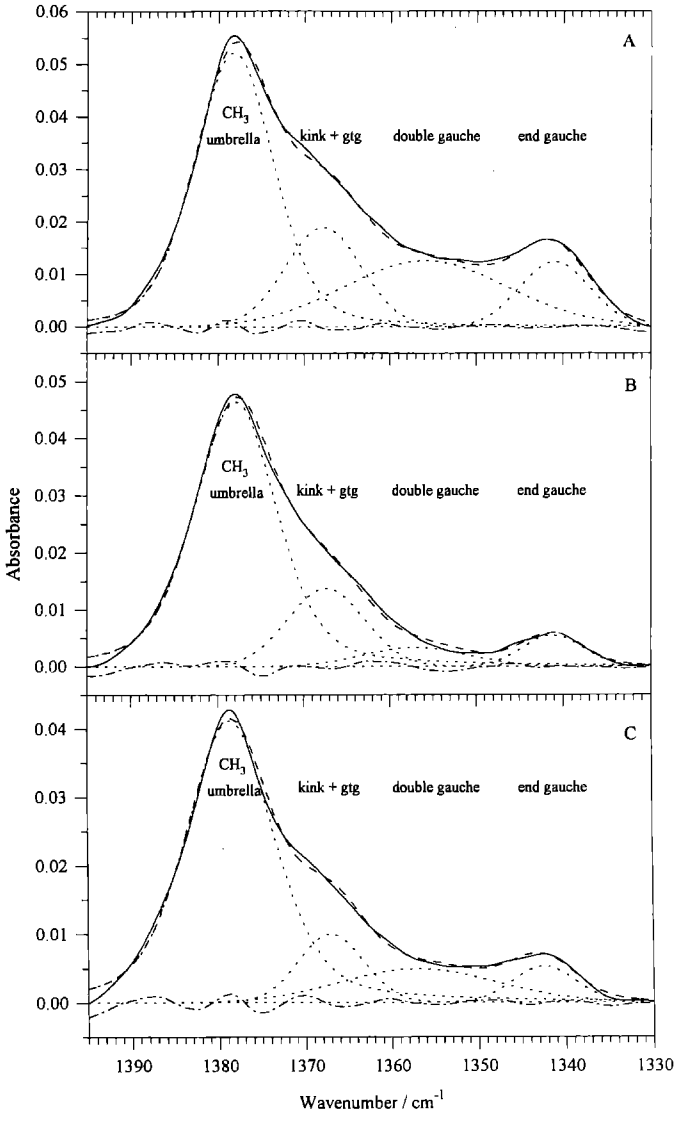

Fig. 2. FT-IR spectra in the low wavenumber region of DMPC **A** DMPE **B** and DMPA (pH 6) **C** at ≈69 °C. Experimental spectrum: *solid line*; total simulated spectrum: *dashed line*; composing bands: *dotted line*; difference between experimental and simulated spectrum: *dash-dotted line*

in frequency. The absolute frequencies determined from the peak maxima depend on the nature of the head group but apparently not in a systematic way. In the liquid-crystalline phase at 60 °C the frequencies are approximately 2922 cm^{-1} with the exception of $DMPG^-$ for which the wavenumber is one cm^{-1} higher. In the gel phase the differences are larger. DMPE and $DMPA^-$ having the highest transition temperatures display the lowest frequencies. Figure 1B shows the effect of a change in head group charge and hydration on the phase behavior and the frequencies. Protonation of $DMPG^-$ leads to the well-known increase in transition temperature (Watts et al. 1978). This is accompanied by a drastic decrease in the frequency of the antisymmetric CH_2-stretching mode in the gel as well as in the liquid-crystalline phase. Deprotonation of $DMPA^-$ to $DMPA^{2-}$ lead to a decrease in the transition temperature (Eibl and Blume 1979) with a concomitant increase in the frequency of the CH_2-stretching vibrations. $DMPA^{2-}$ thus shows a very similar transition curve as $DMPG^-$. The results show that the relative changes agree with the proposition that an increase in conformational disorder causes an increase in wavenumber for the CH_2-stretching vibration. However, these effects cannot be quantified and an exact determination of conformational disorder in the chains is not possible.

We have therefore examined the region of the CH_2-wagging bands to determine the conformational order in the liquid-crystalline phase of the various phospholipids. Representative spectra of DMPC, DMPE, and $DMPA^-$ at ≈69 °C in the region from 1395–1330 cm^{-1} are given in Fig. 2. Superimposed on the experimental curve (solid line) are the results of band simulations of the component bands (dotted line), the sum of the component bands (dashed line) and the difference between experimental and simulated spectra (dash-dotted line). As described in Materials and Methods the experimental spectra were fitted with four Gaussian-Lorentzian functions by a non-linear least square fit. In the fitting procedure the wave numbers of the four bands were only allowed to vary to a certain extent and the intensities, half widths, and proportion of Gaussian component were allowed to vary freely in the iterative fitting procedure. The success of the fitting process depends strongly on the quality of the spectra, particularly the baseline. For that reason at least three different samples of each lipid were prepared and the recorded spectra simulated separately. The 1378 cm^{-1} band is the methyl deformation mode of the methyl groups at the chain ends. The other bands are assigned to specific conformers in the chains as indicated in the figure. From the integral intensities of the individual bands the number of specific conformers and the total number of gauche conformers per chain were calculated using the calibration factors published by Senak et al. (1991).

The FT-IR spectra indicate that the total number of gauche conformers depends on the chemical nature and the charge of the head group of the phospholipid. Figure 3D shows the total number of gauche conformers per acyl chain for phospholipids with different head groups, chain lengths, and head group charge. The total number of gauche conformers is significantly higher for PCs than for the other phospholipids. Between 2.4 and 2.6 gauche conformers per acyl chain are found for phosphatidylcholines in contrast to 1.4–2.0 gauche conformers per acyl chain for PEs, PAs, and PGs.

We compare the conformational properties on an absolute temperature scale because biological systems live at constant temperature. The membrane lipids then have different distances to their respective phase transition temperatures provided that the fatty acyl chains are the same. The observed effect of the different conformational behavior is, however, also present when the data are compared on a reduced temperature scale, though to a somewhat lesser degree. For DPPC at 50 °C, i.e. ~9 degrees above its phase transition temperature, the total number of gauche conformers is reduced from 2.5 at 70 °C to 2.17 at 50 °C (not shown). Thus the total number of gauche for DPPC is still larger than for DPPA at 70 °C, i.e. ~5 degrees above the phase transition. The same applies to the other lipids

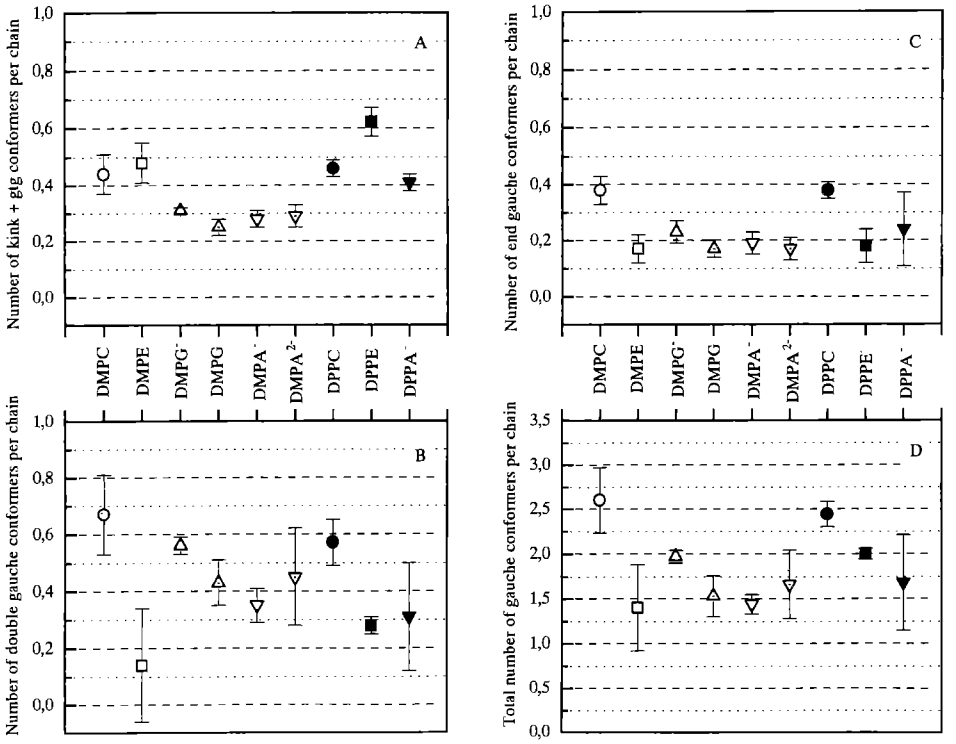

Fig. 3. Number of kink+gtg conformers **A**, number of double gauche conformers **B**, number of end gauche conformers **C**, and total number of gauche conformers per chain **D** for PCs, PGs, PEs and PAs at ≈69 °C

Table 1. Order parameters $\bar{S}_\gamma$, overall number of gauche conformers and number of different conformer sequences of PCs, PEs, PAs, and PGs determined at 69 °C. Standard deviations have been calculated from the results of spectral fits of at least three independent measurements

Phospholipid	Number of kink+gtg sequences	Number of dg sequences	Number of eg sequences	Number of gauche conformers per chain	Order parameter $\bar{S}_\gamma$
DMPC	0.44±0.07	0.67±0.14	0.38±0.05	2.60±0.37	−0.392±0.015
DPPC	0.46±0.03	0.57±0.08	0.38±0.03	2.44±0.14	−0.412±0.006
DMPE	0.48±0.07	0.14±0.20	0.17±0.05	1.40±0.48	−0.442±0.020
DPPE	0.62±0.05	0.28±0.03	0.18±0.06	2.00±0.06	−0.429±0.005
$DMPA^-$	0.28±0.03	0.35±0.06	0.19±0.04	1.44±0.11	−0.440±0.006
$DMPA^{2-}$	0.29±0.04	0.45±0.17	0.17±0.04	1.66±0.38	−0.431±0.015
$DPPA^-$	0.41±0.03	0.31±0.19	0.24±0.13	1.68±0.53	−0.440±0.016
$DMPG^-$	0.31±0.01	0.56±0.03	0.23±0.04	1.97±0.07	−0.418±0.005
DMPG	0.25±0.03	0.43±0.08	0.17±0.03	1.53±0.23	−0.436±0.010

and is also true for the deuterium quadrupole splittings (Hübner and Blume 1987). The differences between phospholipids with different head groups persist even on a reduced temperature scale.

The degree of protonation of PAs and PGs has a slight but measurable influence. The transition temperature of $DMPG^-$ increases upon protonation to 40 °C (Watts et al. 1978). This leads to a reduction of the total number of gauche conformers from 1.97 to 1.53 due to a reduction in electrostatic repulsion between head groups and changes in hydration. Deprotonation of $DMPA^-$, on the other hand, increases the electrostatic repulsion between head groups and decreases the transition temperature (Eibl and Blume 1979). This leads to a slight increase in gauche conformers from 1.44 to 1.66 (see Figs. 2 and 3).

The contributions of the different gauche sequences kink+gtg, double gauche and end gauche to the overall numbers of gauche conformers varies for the different lipids (see Fig. 3 A–C). Despite the relatively large error margins several trends are obvious. The occurrence of kink+gtg conformers is slightly higher in PEs than in PCs, whereas in PCs the chains have more double gauche and particularly end gauche conformers. PAs and PGs have the lowest number of kink+gtg conformers but similar numbers of double gauche and end gauche conformers as PEs. The effect of chain length are negligible and inside the precision of the simulation procedure. The numbers for the different conformers are summarized in Table 1.

^{2}H-NMR spectroscopy

Figure 4 shows as representative examples the ^{2}H-NMR spectra of perdeuterated DMPC and DMPA at 70 °C together with their "de-Paked" spectra calculated for the 90° orientation with respect to the magnetic field (Sternin et al. 1983). The original spectra have relatively low intensities of the shoulders indicating partial orientation of the

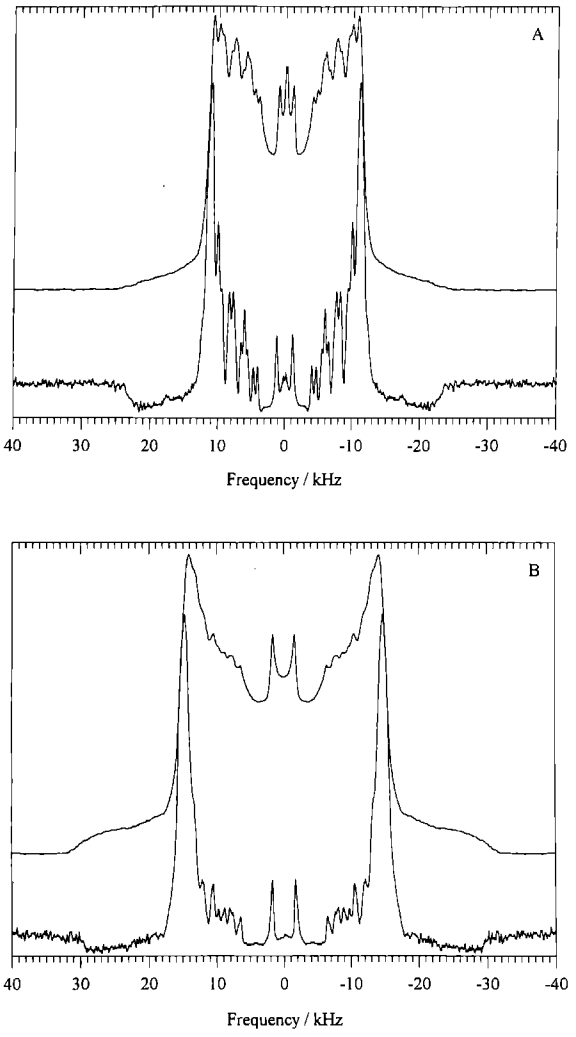

Fig. 4. Experimental ^{2}H-NMR spectra of perdeuterated DMPC **A** and DMPA (pH 7) **B** together with their "de-Paked" patterns calculated for the 90° orientation at 70 °C

bilayers in the high magnetic field (9.39 T). These show up as negative intensities to the left and right of the peaks in the depaked spectra. Similar effects have been observed by Bayerl and Bloom (1990) at lower field strengths and also recently at a field of 9.4 T (Brumm et al. 1992). The partial orientation effects were suggested as being caused by a deformation of the multilamellar vesicles to ellipsoids. The quadrupolar splittings for the different CD_2-segments for the lower part of the chains are well resolved, whereas the splittings from chain positions in the plateau region (carbon number 3–9) are superimposed. The quadrupolar splittings $\Delta\nu_{Q\perp i}$ are obviously much larger for DMPA-d_{54} than for DMPC-d_{54}. They range between 30 and 13 kHz for DMPA-d_{54}, while they are reduced to 22 to 8 kHz in the case of DMPC-d_{54}. In addition, the resonances of the individual chain segments in DMPA are broadened. This is probably caused by a decrease in the transverse relaxation time T_2 with decreasing radius of the multilamellar vesicles. This was found before for DMPC vesicles using ^{2}H-NMR and ^{31}P-NMR spectroscopy (Bayerl and Bloom 1990; Dolainsky et al. 1993). $DMPA^-$, because of the negative charge of its head group, easily forms small vesicles when the sample is vortexed or ultrasonicated. Apparently, even at the high lipid concentration present in the sample used for NMR spectroscopy, the formation of smaller, but probably still multilamellar vesicles cannot be completely avoided. However, the smaller and electrically charged vesicles appear to be less deformable as the orientation effects are less pronounced (see Fig. 4B).

At high pH the formation of small vesicles is even more facilitated as DMPA is doubly charged. In this case isotropic lines of high intensity are observed as soon as the sample is heated to 50 degrees. In the beginning this is not due to hydrolysis of the lipid as checked by TLC but indeed due to the formation of small vesicles with highly curved bilayers. This precludes in this particular case the comparison of the FT-IR results on chain conformers (see above) with the ^{2}H-NMR results.

Taking into account that the resonances of the plateau region are not resolved and the remaining splittings are due to different methylene position in the *sn*-1 and *sn*-2 chain the average deuterium order parameters can be calculated for the different lipids using the relation between quadrupole splitting and order parameter:

$$\Delta\nu_{Q\perp i} = -\frac{3}{4}\left(\frac{e^2qQ}{h}\right)S_{CDi} \qquad (1)$$

and summing over the different chain segments with index *i*. The average order parameter $\bar{S}_{CD}$ is –0.20 for DMPA-d_{54} and –0.135 for DMPC-d_{54}. The same tendency exists for DPPA-d_{62} ($\bar{S}_{CD}$ = –0.19) and DPPC-d_{62} ($\bar{S}_{CD}$ = –0.15).

Discussion

FT-IR spectroscopy: methylene stretching vibrations

The dependence of the methylene stretching vibrations show a certain trend, namely increasing frequencies correspond to decreasing order in the membrane. Recently Kodati and Lafleur (1993) have shown that for POPC/cholesterol and POPE a correlation exists between the center of gravity of the methylene stretching bands and the average deuterium order parameter $\bar{S}_{CD}$. When lipids with differently charged head groups are compared, this correlation obviously does not exist. For instance, the $\bar{S}_{CD}$-value for DMPC at 70 °C is –0.135 compared to –0.185 for DMPE (Marsh et al. 1983), the frequencies for the asymmetric stretching vibration being the same (2922.5 cm^{-1}). $DMPA^-$ has a stretching frequency of 2922 cm^{-1} and an order parameter $\bar{S}_{CD}$ of –0.2. Data on perdeuterated $DMPG^-$ do not exist, but the splittings of specifically deuterated PGs (Garidel and Blume, unpublished) are very similar to those of PCs, the frequencies of their stretching bands being one wavenumber higher (see Fig. 1). The reason for these discrepancies are that not only intermolecular interactions and trans-gauche isomerization affect the stretching frequencies but also the specific position in the chain. Cameron et al. (1981) showed that for specifically deuterated DPPC at low temperature in the gel phase the

frequencies change from 2212 cm^{-1} for the 2-position over 2197 cm^{-1} for the 3-position, 2180 cm^{-1} for the position 7–8 to 2170 cm^{-1} for position 13. This agrees with our observations of frequencies of 2180 cm^{-1} for the 4-, 2169 cm^{-1} for the 6-, and 2171 cm^{-1} for the 12-position of DPPC (Blume et al. 1988; Lehmann and Blume, unpublished). Thus the effects of the position of the methylene group on the vibrational frequency excluding the 2-position (~30 cm^{-1}) far exceeds the effect of an increase in the number of gauche conformers which is only between 3 and 6 cm^{-1} going from the gel to the liquid-crystalline phase. Interestingly, the position effect is smaller on the symmetric CD_2-stretching vibration. Here the frequencies change only by approximately 20 cm^{-1} from position 3 to 13 (Cameron et al. 1981; Blume et al. 1988). It was suggested that the increase in frequency for methylene groups close to the ester group arise from a contribution of the neighbouring C=O dipole (Bansil et al. 1980). If this is the case, then changes in head group charge could also affect the overall frequencies of the stretching vibrations. The observed strong increase in frequency for $DMPA^{2-}$ in the gel phase could support this assumption, as the head group of the PA is now doubly charged. Likewise the decrease in frequency upon protonation of $DMPG^-$ support this notion. Differences in the number of gauche conformers in the gel phases of lipids are usually small so that charge effects should dominate.

The results obtained with lipids with different chemical structures of the head group and the effects observed upon changes in head group charge induced by titration show that the frequencies of the asymmetric and symmetric methylene stretching frequencies can only be used for a specific lipid system as a qualitative indication for the amount of disorder in the liquid-crystalline phase. A comparison of lipids with different head group structure and charge can lead to erroneous results.

FT-IR spectroscopy: methylene wagging bands

The evaluation of the IR wagging bands reveals that the overall number of gauche conformers of PCs is remarkably higher than for PEs and PAs of the same chain length. This phenomenon is mainly due to the high number of double gauche and end gauche conformers in PCs (see Fig. 3A–D and Table 1). $DMPG^-$ has slightly lower number of gauche conformers than DMPC which has almost identical thermotropic behavior. Whereas the methylene stretching band frequency of $DMPG^-$ would indicate higher disorder the opposite is true for the methylene wagging bands. Protonation of $DMPG^-$ shifts the transition temperature to ~40 °C. The large decrease in frequency for the methylene stretching vibration indicates a strong decrease in disorder. This ordering effect is also seen in the methylene wagging vibrations but the effect is not as dramatic. The total number of gauche decreases from ~2.0 to ~1.5 gauche conformers per chain. Deprotonation of $DMPA^-$ on the other hand decreases the transition temperature to 23 °C. The disorder in the liquid-crystalline phase is slightly increased from ~1.45 to ~1.65 gauche conformers per chain. Doubly charged DMPA has the same transition temperature as DMPC but still has a significantly lower number of gauche conformers as DMPC. Despite the almost identical transition temperatures of DMPC, $DMPG^-$, and $DMPA^{2-}$ the chain disorder of these three lipids decreases in the same sequence.

The possibility that vibrational bands stemming from methylene groups of the glycerol backbone or the phosphorylated alcohol are superimposed on the acyl chain wagging bands can be excluded. All investigated lipids contain the glycerol moiety and PCs and PEs both have two methylene groups in their headgroup alcohols. There should either appear some similarity in the spectra of all three lipid classes or at least in the spectra of PCs and PEs. But the spectra of PEs resemble more those of PAs than those of PCs.

Our results for the relative contributions of the different conformational states are in agreement with the data of Senak et al. (1991). In particular, at 70 °C they found a higher number of double gauche conformers for DPPC (0.6) than for DPPE (0.25) and also the number of end gauche conformers is higher for DPPC (0.45) than for DPPE (0.2). These numbers agree well with ours (see Table 1). Difficulties arise in the determination of the number of kink+gtg conformers owing to the overlap of the umbrella vibration of the terminal methyl groups. The absolute numbers therefore depend strongly on the halfwidth and intensity of the umbrella vibrational band. Our numbers for kink+gtg conformers are lower by a factor of approximately 2 than those reported by Senak et al. (1991). The reason for these discrepancies lies in the determination of the integral intensity of the 1368 cm^{-1} band which shows severe overlap with the methyl deformation band (see below). The major problem in data analysis is the method of baseline subtraction in the region of the wagging bands as these bands are superimposed on a sloping background of the asymmetric phosphate vibrational band. We have always subtracted a linear baseline between 1330 and 1395 cm^{-1} (see Materials and methods). Any other baseline taking the sloping background into account would increase the band intensities and thus the numbers for the different conformers.

Molecular dynamics simulations of lipid bilayers and smectic liquid-crystalline phases (van der Ploeg and Berendsen 1982; Egberts and Berendsen 1988; Pastor et al. 1988a, b) have shown in agreement with statistical mechanical models (Meraldi and Schlitter 1981a, b) that kinks have a relatively low contribution to the total disorder of the chains, namely only between 0.2 in 0.5 kinks occur in a C_{10} or C_{16} chain, respectively. Thus the low number of kinks and gtg conformers we determined by our simulations does not seem to be unreasonable.

For DPPC, Mendelsohn et al. (1989, 1991) investigated the conformational isomerism by analysis of the CD_2 rocking mode absorptions for compounds specifically deuterated at positions 2, 3, 4, 6, 10, 12 and 13 of the acyl chains. By combination with calculated numbers for conformers by Meraldi and Schlitter (1981a, b) they determined an average number of gauche conformers of 3.6–4.2 per chain, a value which is similar to the results of Senak et al. (1991), determined by the analysis of the wagging bands (3.2–4.0 gauche conformers).

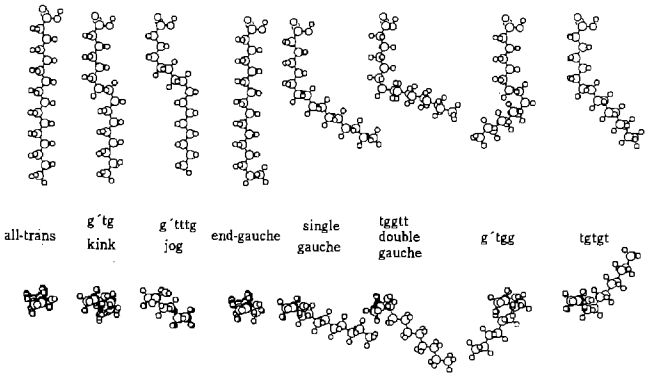

Fig. 5. Illustration of different possible conformers in aliphatic lipid chains viewed from the side and from the top to illustrate the sideways displacement of the chain

As a consequence of the lower number of kinks and gtg conformers in our simulation the total number of gauche conformers we report here is also lower, namely 2.5 for DPPC at ≈69 °C. Again, a slight decrease in the integral intensity of the umbrella vibrational band would increase these numbers owing to an increase in the number of kinks and gtg conformers. We think, however, that the lower number of total gauche conformers we determined here is not unreasonable because of the following reason. The determination of gauche conformers from the wagging bands does not take into account the single gauche conformers and the jogs, i.e. those chain conformations where a g^+ and g^- conformation is separated by at least three trans segments (see Fig. 5). The contribution of these conformers cannot be neglected as the calculations of Meraldi and Schlitter (1981 a, b) and the experimental results of Mendelsohn et al. (1989, 1991) show. Jogs would probably contribute 0.4 gauche conformers per chain, while up to one single gauche per chain might be present. Thus the total number of gauche conformers could increase to ~3.4 to 3.9. Pastor et al. (1988 a, b) have recently reported a molecular dynamics simulation of a C_{16} chain in a bilayer and have been able to reproduce the order parameters of DPPC using two different approaches. Without incorporation of chain wobbling they determined ~4.3 gauche per chain. With inclusion of chain wobbling this number was reduced to ~3.65. The latter number is well within the limit of our estimations.

Because of the difficulties in determining absolute numbers for different conformers we want to discuss in the following only the differences in the number of conformers between the phospholipids with the different head groups.

Apparently, the headgroup of PCs allows a less dense arrangement of the acyl chains as compared to PEs and PAs. The double gauche conformers and, of course, the end gauche conformers occur in the interior of the double layer where its two leaflets are in contact. The x-ray crystallographic data of these lipids (Pascher et al. 1992) show that their glycerol moiety is arranged parallel to the bilayer normal in the case of PCs and PEs, whereas it is oriented perpendicular to the bilayer normal in the case of PAs. The arrangement of the glycerol moiety parallel to the bilayer normal requires a bend in the *sn*-2 chain in order for the two aliphatic chains to align parallel. For that reason, the *sn*-2 chain cannot extend as deep into the bilayer interior as the *sn*-1 chain, thereby creating free volume in the bilayer interior. Probably, the orientation of the glycerol moiety in crystalline PCs is conserved in the liquid crystalline phase. The glycerol backbone of PEs seems to change from a parallel to a more perpendicular orientation with respect to the bilayer normal on dispersing the crystalline lipid in water. As a consequence, the *sn*-1 and *sn*-2 chains of PEs and PAs can extend almost equally deep into the bilayer interior, leaving not as much space for double gauche and end gauche conformers. This reasoning is supported by the order parameter profiles obtained via ^{2}H-NMR spectroscopy. The quadrupolar splittings $\Delta\nu_{Q\perp i}$ of PCs reach values of about 8 kHz near the methyl end of the acyl chains, whereas the minimum quadrupolar splittings of PEs and PAs are approximately 14 kHz (see Fig. 4 and Seelig and Seelig 1974; Marsh et al. 1983; Thurmond et al. 1991; Blume et al. 1982).

An alternative view of the headgroup influence on the conformational freedom of the acyl chains yields a similar explanation. The static picture of the orientation of the glycerol backbone is certainly simplified. An orientation of this moiety as observed in the crystal structures should result in equal quadrupolar splittings for phospholipids being deuterium labeled at the 1, 2 or 3 position of the glycerol moiety. However, Gally et al. (1975, 1981) and Strenk et al. (1985) found different splittings for the deuterons in 1 and 3 position of PCs and other phospholipids. These spectroscopic findings have been explained in two ways. The glycerol moiety either has a rigid trans conformation but its orientation with respect to the long molecular axis is different from that in the crystal structures or the glycerol moiety can undergo trans-gauche isomerizations around the glycerol C_1–C_2 and C_2–C_3 bonds. Blume et al. (1982) observed for DPPE labeled at the 2 position of the glycerol moiety a quadrupolar splitting of 95 kHz in the gel phase at 55 °C. This value is inconsistent with the glycerol orientation observed in the crystal structure. In the liquid crystalline phase the splitting reduces to 26 kHz indicating another change in orientation and/or conformation of the glycerol backbone. The idea of fast transitions between different conformations is supported by potential energy calculations by McAlister et al. (1973). These authors found that the trans and the gauche conformation with respect to the C_2–C_3 bond of the glycerol moiety are of comparable energy. Schindler and Seelig (1975) as well as Meraldi and Schlitter (1981 b) had to combine different conformational sequences for the first segment of the acyl chains in order to adapt their model calculations to the experimental order parameter profile. Since these order parameters were interpreted in terms of trans-gauche isomerization alone, the assumption of different conformations for the first segment may be indicative of dynamic processes within the glycerol backbone and also of angular fluctuations of the entire acyl chains.

There seem to exist rapid equilibria between different glycerol conformations of phospholipids, the average orientation of the glycerol moiety being neither perpendicular nor parallel to the bilayer plane. These motions have

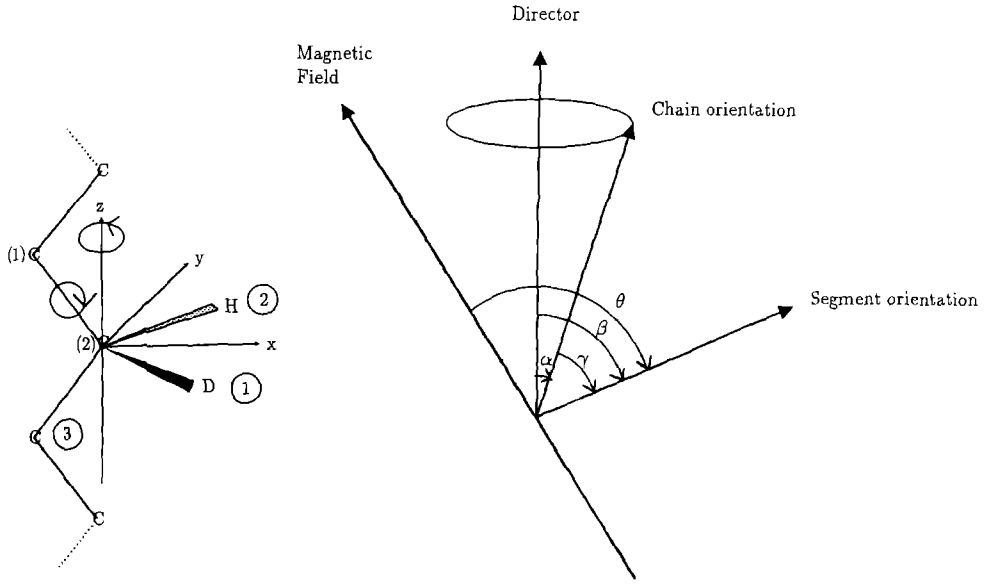

Fig. 6. *Left:* Representation of an aliphatic chain denoting the possible positions of a deuteron on trans-gauche isomerization. *Right:* Vectors and angles describing the reorientational motions in lipid chains according to Petersen and Chan (1977). The director is perpendicular to the bilayer surface. The segment orientation coincides with the C–D bond vector

been extensively studied by ^{2}H-NMR for a glyceroglycolipid and a phospholipid analogue ^{2}H-labeled at the C_3 position of the glycerol backbone (Auger et al. 1990a, b; Auger and Jarrell 1990). These authors found that the intramolecular motions in the glycerol backbone even persisted down to low temperatures where the lipid was in the gel state.

The large size of the PC headgroup that is responsible for the chain tilt in the gel phase, will allow tumbling motions and trans-gauche isomerizations of the glycerol backbone in the liquid crystalline phase, thereby causing the acyl chains to shift with respect to each other. Such a motional mode will also give rise to free volume in the membrane interior. The headgroup region of PEs and PAs is more rigid than the PC headgroup owing to the immobilization brought about by an extensive hydrogen bonding network that is not possible in PCs. In addition, the described motional modes of the glycerol backbone are influenced by the extent of hydration which is higher for PCs. The higher conformational order is thus a direct consequence of headgroup structure and mobility.

Comparison of FT-IR and ^{2}H-NMR-results

In order to interpret the lineshapes and quadrupolar splittings in the ^{2}H-NMR spectra and the number of gauche conformers calculated from the integral intensities of the IR wagging bands, it is necessary to relate these quantities to the different motions executed by phospholipids in model membranes. The observed lineshapes and splittings in the ^{2}H-NMR spectra are sensitive to (Petersen and Chan 1977):

a) long axis rotation of the entire acyl chains,
b) trans-gauche isomerization around C–C bonds,
c) restricted wobbling motions of the lipid molecules,
d) fast lateral diffusion around highly curved surfaces in small vesicles and rotational tumbling of small vesicles.

Excluding the motions of the bilayer aggregates and the lateral diffusion summarized under d) all other motions of lipid molecules are in the fast limit on the ^{2}H-NMR time scale as long as the lipids are in the liquid crystalline L_α phase and thus contribute to the reduction of the quadrupole splittings. In addition, they are axially symmetric with respect to a director oriented perpendicularly to the membrane surface. The observed time-averaged signals are normally interpreted in terms of the order parameter concept (Maier and Saupe 1958; Seelig 1977; Petersen and Chan 1977).

The order parameter S_{CDi} depends on the time-averaged orientation of the C–D bond vector with respect to the molecular long axis (Seelig 1977):

$$S_{CDi} = \frac{1}{2}\overline{(3\cos^2\vartheta - 1)}; \tag{2}$$

with ϑ being the angle between the C–D bond vector and the molecular long axis.

We consider an aliphatic chain with the z-axis being the molecular long axis. The numbering of the possible positions of the deuterons is depicted in Fig. 6. The probabilities of deuteron number 1 to occupy the positions 1, 2 and 3 shall be p_1 (trans, ϑ=90°), p_2 (gauche$^-$, ϑ=90°) and p_3 (gauche$^+$, ϑ=35.5°), the sum of all probabilities being equal to 1:

$$\sum_{j=1}^{3} p_j = 1. \tag{3}$$

For a deuteron in position number 2 a similar expression holds. For the order parameter S_{CDi} we obtain from Eqs. (2) and (3):

$$S_{CDi} = \frac{1}{2}\sum_{j=1}^{3} p_j(3\cos^2\vartheta_j - 1), \tag{4}$$

and by inserting the two possible values of 35.5° and 90° for ϑ_j:

$$S_{CDi} = \frac{1}{2} - (p_1 + p_2) = p_3 - \frac{1}{2}. \tag{5}$$

The quadrupolar splitting $\Delta\nu_{Q\perp i}$ of a CD_2-segment in the case of fast anisotropic motion about the molecular long axis is related to the order parameter S_{CDi} by (Seelig 1977):

$$\Delta\nu_{Q\perp i} = -\frac{3}{4}\left(\frac{e^2qQ}{h}\right)S_{CDi}. \quad (6)$$

By inserting a value of 167 kHz for the quadrupole coupling constant ($e^2\,q\,Q/h$) of an aliphatic C–D bond and by combining Eqs. (5) and (6), we obtain

$$\Delta\nu_{Q\perp i} = 62.5 \cdot p_1 = 62.5 \cdot (1 - 2\,p_3) \text{ all-trans chain.} \quad (7)$$

Thus, a fast rotating, rigid leads to a reduced quadrupolar splitting $\Delta\nu_{Q\perp i}$ of 62.5 kHz ($p_3 = 0$, $S_{CDi} = -1/2$). Fast trans-gauche isomerizations in the chain then leads to a further decrease of the quadrupolar splitting according to Eq. (7).

The quadrupolar splittings $\Delta\nu_{Q\perp i}$ are accessible from ^{2}H-NMR spectra of phospholipids with perdeuterated acyl chains or from spectra of lipids with specifically deuterated chains. To be able to compare the ^{2}H-NMR data with the FT-IR data we use the arithmetic mean of the order parameters S_{CDi} of all CD_2 segments, i.e. the average order parameter $\bar{S}_{CD}$. This average order parameter can also be determined from the first or second moments (Davis 1983). This was not possible in our case because of the partial orientation of the bilayers in the magnetic field (see above).

In reality, the average order parameter $\bar{S}_{CD}$ does not depend on the conformational probabilities alone, as is suggested by Eqs. (5) and (7), but implicitly contains information on the extent of other fast reorientational motions, for instance, the restricted wobbling motions of the chains.

Petersen and Chan (1977) divided S_{CD} into two contributions arising from order parameters S_α and S_γ defined in the usual way with angles α and γ shown in Fig. 6:

$$S_{CD} = S_\alpha \cdot S_\gamma, \quad (8)$$

S_α describes the angular deflections of the molecular long axis with respect to the director axis, while S_γ represents exclusively the conformational order with respect to the instantaneous orientation of the molecular long axis. However, the definition of the orientation of molecular long axis is a problem. The FT-IR results show that besides kinks, jogs, and gtg conformers, also single and double gauche conformers are present in the chains. These are shown in Fig. 5. Depending now at which segment the position of the gauche or double gauche conformer is located, the length of the more or less extended part of the chain is either at the beginning of the chain or below the gauche bond. Thus the long molecular axis can be defined as either lying below or above this gauche conformation or, if the single gauche appears in the middle of the chain, even somewhere in between. The long molecular axis can, of course, be arbitrarily fixed in the most rigid part of the molecule, namely the first two segments of the *sn*-1 chain. In the case of a single gauche conformation at the bond between C_3 and C_4 for all CH_2-groups below starting with segment 4 an order parameter S_γ of +1/2 for one of the deuterons and –1/2 for the other would be calculated. If the occurence of all possible conformational isomers for each of the individual C–C bonds were known from the experiments, then the segmental order parameters S_γ could be calculated. This is, however, not the case and thus precludes a quantitative separation of the two effects causing the reduction in the quadrupole splittings, namely the chain wobbling motion and the trans-gauche isomerization around C–C bonds.

We have attempted to get at least some qualitative insight by defining an average order parameter $\bar{S}_\gamma$ which is calculated from the number of gauche conformers per chain determined by FT-IR spectroscopy, neglecting all the problems mentioned above and assuming that the chain above the gauche conformations always defines the direction of the molecular long axis. On the assumption that gauche$^+$ and gauche$^-$ conformers are equally probable, this average order parameter $\bar{S}_\gamma$ in our definition is related to the average probability $\bar{p}_3$ of gauche conformers by simply using the same relation as for S_{CD}, namely:

$$\bar{S}\gamma = \bar{p}_3 - \frac{1}{2}, \quad (9)$$

with

$$\bar{p}_3 = \frac{n_{\text{gauche}}}{2 \cdot n_{CH_2}}. \quad (10)$$

n_{gauche} is the number of gauche conformers per acyl chain as determined via spectral simulation of the IR-wagging bands and n_{CH_2} is the number of methylene groups per acyl chain. Division of $\bar{S}_{CD}$ by $\bar{S}_\gamma$ then yields the average acyl chain order parameter $\bar{S}_\alpha$, representing chain wobbling motion only. *$\bar{S}_\alpha$, $\bar{S}_{CD}$, and $\bar{S}_\gamma$ will be used solely for comparing the different lipid systems. These order parameter have no direct physical meaning.*

Previously, the analysis of the ^{2}H-NMR order parameter profiles and their simulation was solely based on the assumption that wobbling motions of the long molecular axis are absent and do not contribute to the reduced quadrupole splittings (Seelig and Seelig 1974; Schindler and Seelig 1975; Meraldi and Schlitter 1981 a, b). With this assumption the order parameter profiles could be simulated quite satisfactorily. However, ^{2}H-NMR T_1 and T_2 relaxation time measurements have shown that wobbling motions of the molecular long axis with correlation times of $\sim 10^{-8}$ s are present in liquid-crystalline bilayers (Mayer et al. 1988, 1990) and lead to additional averaging and reduction of the ^{2}H-NMR quadrupolar splittings. Recently, Pastor et al. (1988 a, b) have included the wobbling motions in their molecular dynamic simulations and their calculations for T_1 relaxation times in lipids bilayers.

To obtain information on the importance and relative contributions of chain wobbling motions we used the Petersen & Chan model (Petersen and Chan 1977) and calculated averaged $\bar{S}_\alpha$ values from the combination of our FT-IR and ^{2}H-NMR results. The results are shown in Table 2.

The chain wobbling motion and thus the order parameter $\bar{S}_\alpha$ of the lipid molecules depends on the differences in headgroup size and interactions. This motional mode, information on which is implicitly contained in the order parameter $\bar{S}_{CD}$ determined from the ^{2}H-NMR "de-Paked" patterns, is somewhat more pronounced in the case of PCs.

The order parameter $\bar{S}_\alpha$ is a purely operational quantity for two reasons: The first is the problem of defining

Table 2. Comparison of the averaged deuterium order parameters $\bar{S}_{CD}$ the averaged segmental order parameter $\bar{S}_\gamma$ and the averaged calculated wobbling order parameter $\bar{S}_\alpha$ for phospholipids with different head groups and chain lengths. The standard deviation for the order parameter $\bar{S}_\alpha$ contains only errors from $\bar{S}_\gamma$. $\bar{S}_\alpha$, $\bar{S}_{CD}$, and $\bar{S}_\gamma$ are used solely for comparing the different lipid systems. These order parameters have no direct physical meaning

Lipid	$\bar{S}_{CD}$	$\bar{S}_\gamma$	$\bar{S}_\alpha$
DMPC	–0.135	–0.392±0.015	0.344±0.014
DPPC	–0.15	–0.412±0.006	0.364±0.005
DMPE	–0.185[a]	–0.442±0.020	0.419±0.019
DPPE	–0.194[b]	–0.429±0.005	0.452±0.005
DMPA	–0.20	–0.440±0.006	0.455±0.007
DPPA	–0.19	–0.440±0.016	0.432±0.016

[a] Calculated from data by Marsh et al. (1983)
[b] Calculated from data by Thurmond et al. (1991)

the directions of the molecular long axis. The second is that the total number of gauche conformers determined by FT-IR spectroscopy does not include all conformational states that are theoretically possible (see above). The single gauche state, for example, plays an important role in rotational isomerism according to model calculations by Meraldi and Schlitter (1981 a, b). They calculated a probability of 12–20% for the occurrence of a single gauche conformer (g, 60°) in the acyl chains of DPPC (the different conformations are characterized by their conformational state (gauche or trans) and by the angle between the normal to the membrane surface and the vector connecting the midpoints of two consecutive C–C bonds). This conformational state was further classified with respect to the number of trans conformers (t, 60°) that follow the gauche bond. Approximately 50% of the single gauche conformers (g, 60°) are followed by only one trans conformer (t, 60°). Therefore, most single gauche states belong to the kink+gtg conformer sequence and are taken into account in the evaluation of the wagging bands. Nevertheless, the total number of gauche conformers is underestimated on the basis of the FT-IR data and thus the $\bar{S}_\gamma$ values are too high. Including thus additional single gauche conformers and jogs reduces $\bar{S}_\gamma$ and will increase the wobbling order parameter $\bar{S}_\alpha$ to a likely value. Pastor et al. (1988b) found good agreement between calculated T_1 relaxation values and experiments for $\bar{S}_\alpha$ values between 0.5 and 0.7.

On the other hand, trans conformers (t, 60°) following a single gauche (g, 60°) are interpreted as gauche states within the framework of our theoretical model relating the quadrupolar splitting to the probability of a C–D bond to occur at a certain angle to the molecular long axis. In contrast, gauche conformers of the (g, 0°) type count as trans states in our theoretical approach. Considering the probabilities of these conformations and their interdependence (Meraldi and Schlitter 1981 a, b), both effects will partially compensate. The number of gauche conformers determined from the quadrupolar splittings using equation 7 is slightly overestimated. For instance, for the 4-position of DPPC a gauche probability of 0.3 is calculated by Meraldi and Schlitter (1981 a, b) using the ^{2}H-NMR data. FT-IR spectroscopy of specifically labeled DPPC yields a value of only 0.21 (Mendelsohn et al. 1989). It should be noted, however, that the relative probabilities of the different conformers deduced from hard core repulsion theory by Meraldi and Schlitter (1981) depend on the conformation of the first segment of the acyl chains, on the chosen lateral pressure, and on the assumption that wobbling motions are absent, i.e. $S_\alpha = 1$.

We will now compare the different $\bar{S}_\alpha$ values obtained for PCs, PEs and PAs. $\bar{S}_\alpha$ is lowest for PCs, indicating that orientational fluctuations of the molecular long axis are present to a higher degree in this lipid class than in PAs and PEs. This is in accord with the above mentioned headgroup effects that already explained the enhanced trans-gauche isomerizations. PAs and PEs behave very similar in this respect. In this context, it is important to note that PEs have a slightly higher number of kink+gtg conformer sequences. There appear to be several different ways by which the lipid molecules preferably fill out the volume at their disposal. The situation of PCs is clear in that they possess the highest extent of trans-gauche isomerizations as well as orientational fluctuations of the long molecular axis due to their head group nature. Much more subtle are the effects in the case of PEs and PAs. Both lipid classes have almost the same overall number of gauche conformers, but different gauche conformer sequences are not equally abundant. Fluctuations of the long molecular axis are more or less the same. The very similar motional behavior of PAs and PEs agrees with their almost identical thermodynamic transition parameters. Head group size, head group hydration, head group interactions via hydrogen bonds and optimal van-der-Waals interactions in the hydrophobic membrane core are in a very sensitive balance and stipulate the differences in motional behavior of the different lipid classes.

The present analysis relies on averaged order parameters, which have no direct physical meaning. Still, this approach is useful in determining differences in conformational behavior between the various phospholipid classes. A more detailed and more informative method is the analysis of the rocking vibrations of CD_2 groups of specifically labeled lipids (Mendelsohn et al. 1989, 1991; Davies et al. 1992). For DPPC and DPPE labeled at the 4-position not only differences in the total probability for a gauche conformation was observed, DPPC having the higher probability, but also differences in the distribution of conformers. For DPPE ≈ 80% are multiple gauche forms at this particular chain position, whereas for DPPC ≈ 85% belong to the kink and single gauche class (Mendelsohn et al. 1989; Davies et al. 1992). The relative contributions of different conformer classes to the total number of conformers is, of course, eliminated when average order parameters are used. A more thorough analysis has therefore to resort to specifically labeled phospholipids. Studies using specifically labeled DPPA to resolve these differences are under way.

Summary

The analysis of the methylene stretching vibrations of phospholipids in the gel as well as liquid-crystalline phase

show that the frequencies of these bands can only be used as a qualitative indication for the amount of chain disorder. A more quantitative estimate can be obtained by the analysis of the methylene wagging vibrations. The total number of gauche conformers and the contribution of different conformer classes depends on the nature of the phospholipid head group.

Merging FT-IR with ^{2}H-NMR spectroscopic results allows an analysis of the importance of the different motional modes of phospholipids. Previous analyses and simulations of the deuterium order parameters have in most cases used the assumption that no wobbling motions occur. The comparison of the order parameters $\bar{S}_{CD}$ (^{2}H-NMR spectroscopic results) with $\bar{S}_\gamma$(FT-IR spectroscopic results) show that wobbling motions are present in lipid bilayers. Our data allow an estimate of the extent of orientational fluctuations of the molecular long axis in different phospholipids. Phosphatidylcholines show the highest number of trans-gauche isomerizations and slightly more chain wobbling motions as compared to phosphatidylethanolamines and phosphatidic acids.

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