Comparison between orientational and conformational orders in fluid lipid bilayers

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ABSTRACT The orientational order as determined by ^{2}H NMR and the infrared frequencies of the C—H stretching modes of the methylene groups have been measured for several systems (POPC, POPC/cholesterol and POPE), all in the fluid phase, and then were compared; this work reveals an unexpected linear correlation between them. This experimental result shows that both measurements are essentially sensitive to a common motion, most likely *trans/gauche* isomerisation. This new correlation with those already found in the literature suggest that several measurements related to the hydrophobic core of the fluid bilayer describe different aspects of a universal behavior. The correlation presented here does not extend to the lipid in gel phase where slower motions affect the NMR lineshape.

INTRODUCTION

The hydrophobic core of the bilayer formed by the stacked lipid acyl chains is more flexible than the rigid lipid headgroup. For example, ~90% of the entropy change associated with the gel-to-liquid phase transition is attributed to the disordering of the acyl chains (Phillips, 1972). The sensitivity of the apolar core to the composition of the bilayer and to external conditions provides useful insights into the influence of various parameters on membranes. The order of the acyl chains is indeed related to lipid phase (Mantsch and McElhaney, 1991). In addition the acyl chain order has also been related to several important parameters of the membrane such as its thickness (Seelig and Seelig, 1974; Ipsen et al., 1990), its polymorphic tendencies (Lafleur et al., 1990a), and the solubility of apolar species (De Young and Dill, 1988). At this point, it is important to have a quantitative description of the order of the bilayer apolar core to develop models describing the membrane behavior. As a consequence, the quantitative characterization of the acyl chain order of lipid bilayers has been an important goal for several spectroscopic techniques (for a general review, see Gennis, 1989). In this paper, our work has been focused on the orientational order as measured by deuterium nuclear magnetic resonance (^{2}H NMR), and on the conformational order as measured by infrared (IR) spectroscopy.

^{2}H NMR spectroscopy is a powerful technique to investigate lipid chain order (for general reviews, see Davis, 1979, 1983). The quadrupolar splitting is directly measurable on the spectrum of lipids bearing one or several deuteriated methylene groups; it originates from the interactions between the quadrupole moment of the deuterium nucleus and the electric field gradient whose principal axis is along the C—D bond. The strength of these interactions depends on the angle between the C—D bond and the external magnetic field. Molecular motions affecting this angle lead to the averaging of the quadrupolar interactions. In the fluid lipid phase, the rotation of the lipid along its long axis is fast on the NMR time scale (10^{-5} s), and the system shows axial symmetry. When measured for a known orientation of a fluid lipid bilayer relative to the magnetic field, the quadrupolar splitting is then a direct measurement of the intramolecular averaging of the quadrupolar interactions; it is simply related to the orientational order parameter by:

$$\Delta\nu_Q = \tfrac{3}{4}A_q S,$$

where $\Delta\nu_Q$ is the quadrupolar splitting measured for a bilayer whose normal is perpendicular to the external magnetic field direction, A_q the quadrupolar coupling constant ($\approx$167 kHz), and S the orientational order parameter.

Infrared spectroscopy has also been widely used to characterize lipid chain order, especially since Fourier transform infrared spectroscopy (FTIR) has been available, providing better convenience for working in aqueous medium. The information on the lipid acyl chains has been mainly extracted from the frequency maximum and bandwidth of the C—H stretching (ν_{C-H}) vibrations (for a review, see Mantsch and McElhaney, 1991). These vibrations are related to localized bonds which are not directly involved in the isomerisation of the carbon backbone, but appear to be sensitive to chain order. One of the pioneer papers in this area was published by Asher and Levin (1977). The authors studied the infrared spectra of a variety of lipid/water assemblies as a function of temperature. It appeared that the frequency of the bands associated with the CH_2 symmetric and antisymmetric stretching modes observed at 2,850 and 2,920 cm^{-1}, respectively, could be useful to monitor chain order since there was a sharp band shift of ~3 cm^{-1} concomitant with the gel-to-liquid crystalline phase transition. The authors stated that "these frequency shift parameters sensitively monitor conformational changes arising from temperature dependent intermolecular effects". In a parallel manner, Cameron et al. (1979) determined the melting curve of dipalmitoylphosphatidylcholine (DPPC), using the change of the integrated intensity of the antisymmetric C—H stretching as a function of temperature. These seminal works

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have promoted a considerable number of studies on lipids using FTIR. The technique has been applied successfully to the investigation of the gel-to-liquid crystalline phase transition, the L_α to H_{II} phase transition (Mantsch et al., 1981), and DPPC pretransition (Cameron et al., 1980). Casal et al. (1980, 1982) proposed that the frequencies of the CH_2 stretching modes (as well as the CD_2 groups) are essentially related to the *trans/gauche* isomerisation, and that "other motional effects not resulting in the introduction of gauche conformers do not produce frequency shifts". The effect of temperature and cholesterol on these spectral parameters compared with other spectroscopic measurements (mainly ^2H NMR and Raman spectroscopy) supported this proposition, and it has become a generally accepted statement. However, one should keep in mind that this proposition is inspired from comparative arguments, and no quantitative analysis has been made yet.

More recently, two new methods have been proposed to determine quantitatively the conformational order of the lipid acyl chains. One method is based on the analysis of the CH_2 wagging region (1,300–1,400 cm^{-1}). These vibrational modes give rise to well resolved bands assigned to methylene groups participating in the different chain conformations such as end gauche, kinks, and double gauche (Snyder, 1967). The "concentration" of individual conformer over the whole lipid chains can be estimated from the integrated intensity of each band. No distinction can be made relative to the chain position (sn1 versus sn2 chains) or to the carbon position along the chains. This method has been successfully applied to the study of sodium dodecyl sulfate micelles (Holler and Callis, 1989) and phospholipid bilayers (Casal and McElhaney, 1990; Senak et al., 1991). The other IR method proposed to characterize the chain order in a quantitative manner is based on the analysis of the CD_2 rocking modes between 660–600 cm^{-1} (Mendelsohn et al., 1989). The frequency of these bands is sensitive to the local geometry of the neighboring carbon bonds. The analysis of this region allows the calculation of the fraction of gauche conformers at a specific position. This method is not being examined in this work.

The study presented in this paper was motivated by the desire to compare the orientational order measured by ^2H NMR and the conformational order measured by IR parameters in fluid bilayers. We have modulated the order of the apolar core of fluid bilayers by varying three parameters: temperature, headgroup, and cholesterol content. These factors have been selected for two reasons. First, their nature is different, so the modulation of the lipid chain order has different origin (Lafleur et al., 1990b). Temperature is a physical parameter; an increase of temperature excites more conformers and then, affects the chain order. The change of the headgroup corresponds to a chemical modification at the bilayer apolar core/water interface. Since phosphatidylethanolamine and phosphatidylcholine show a different amphiphilic balance, the packing of the lipid chains (and thus their order) is affected. The presence of cholesterol corresponds to the addition of an extra component to the system. The ordering effect of cholesterol on lipid chains in the liquid crystalline phase is well established (Umemura et al., 1980; Stockton and Smith, 1976; Vist and Davis, 1990), and has been attributed to the smooth surface of cholesterol promoting the stiffening of the lipid chains (Bloom et al., 1991). The second reason for this choice of parameters is the fact that they had already been characterized by ^2H NMR (Lafleur et al., 1990b).

MATERIALS AND METHODS

All the phospholipids were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and cholesterol was obtained from Sigma Chemical Co. (St. Louis, MO). Separate lipid and cholesterol stock solutions were prepared by dissolving each in a benzene/methanol (96/4% (vol/vol)) solution. The various phospholipid/cholesterol mixtures were prepared by mixing a constant volume of lipid solution and the appropriate amounts of cholesterol solution. Then the mixtures were freeze dried under vacuum overnight.

The samples for the IR study were prepared by suspending the freeze dried lipid mixtures in a 20 mM Hepes buffer containing 100 mM NaCl and 5 mM EDTA (pH = 7.4) in order to obtain a final phospholipid concentration of 12 (wt/vol)%. The samples were then vortexed in the fluid phase to ensure complete hydration. The sample was placed between CaF_2 windows with a Teflon spacer of 5 μm thickness. The temperature of the brass sample holder was controlled by circulating water. The temperature was monitored with a thermocouple next to the sample. The spectra were recorded at 2 cm^{-1} resolution using Nicolet FTIR 5DXB spectrometer. 1,000 interferograms were coadded and Fourier transformed using the Happ-Genzel function for apodization. The ν_{C-H} region was corrected for the strong water absorption (ν_{O-H} ~ 3,400 cm^{-1}) using a fourth degree polynomial fit. The center of gravity and the width of the bands were determined using algorithms described elsewhere (Cameron et al., 1982). The reproducibility of these measurements has been found to be ~0.2 cm^{-1}.

A similar procedure has been used for the ^2H NMR samples, except that phospholipids bearing a perdeuteriated palmitoyl chain have been used (for the details, see Lafleur et al., 1990b). The spectra have been recorded on a 46 MHz ^2H NMR home-built spectrometer already described (Davis, 1979; Sternin, 1985). The spectra were obtained using a quadrupolar echo pulse sequence with phase cycling. The 90° pulse length was 4 μs, and the interpulse delay was 50 μs. Free induction decays were acquired in quadrature, collecting 4,096 points with a 5 μs dwell time. The delay between successive pulse sequences was at least 300 ms, and at least 25,000 FIDs were coadded. The order profiles were obtained from the powder pattern spectra using the method described by Lafleur et al. (1989). The average order parameter, $\langle S \rangle$, corresponds to the arithmetical mean of the values of $S(n)$ for $2 \leq n \leq 16$ [$S(16)$ was linearly extrapolated from $S(14)$ and $S(15)$ since the different symmetry of the terminal methyl leads to a reduced $S(16)$].

RESULTS

Fig. 1 shows typical infrared spectra of lipid systems in the C—H stretching region (*left*) and in the CH_2 wagging region (*right*). The spectra were obtained (*A*) from 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) dispersion (0°C), (*B*) cholesterol in KBr (room temperature), and (*C*) POPC/cholesterol 45 (mol) % mixture (0°C). For the phospholipid, the symmetric and anti-

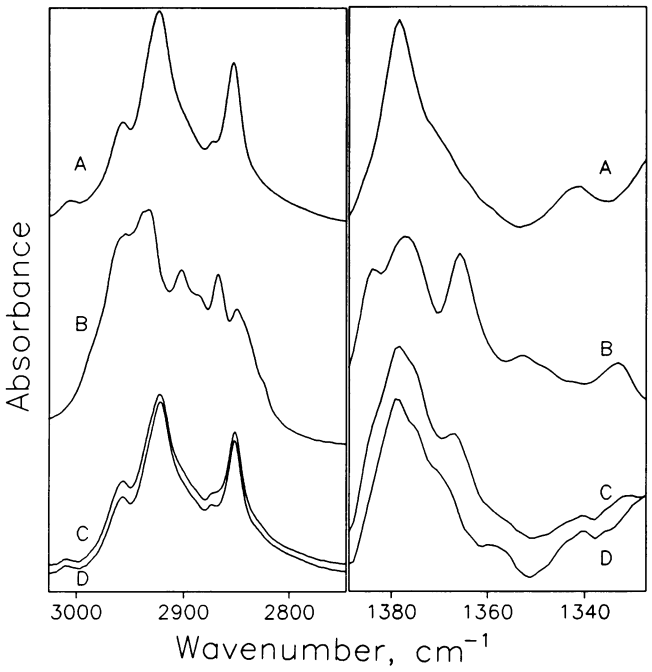

FIGURE 1 FTIR spectra in the C—H stretching (*left*) and CH_2 wagging (*right*) regions of (*A*) POPC (0°C), (*B*) cholesterol (room temperature), and (*C*) POPC with 45 (mol) % cholesterol (0°C). *D* shows the spectrum (*C*) corrected for cholesterol contribution.

symmetric C—H stretching of the methylene groups are found at 2,851 and 2,919 cm^{-1}, respectively (Asher and Levin, 1977). The symmetric and asymmetric C—H stretching of the terminal methyl groups are observed at 2,875 and 2,956 cm^{-1}, respectively. Finally, the =C—H stretching of the oleoyl chain is observed at 3,012 cm^{-1} (Mantsch et al., 1981). In order to isolate the phospholipid bands in POPC/cholesterol mixtures, the spectra have been corrected for the contribution of cholesterol. Pure cholesterol spectra have been obtained from a benzene solution as well as from KBr pellet; no significant differences were observed between these spectra. Since cholesterol solubility in water is very low, it was impossible to record a spectrum from a homogeneous cholesterol/water sample. The spectrum obtained from such a sample showed a similar profile as those recorded from KBr pellet or benzene solution, but the bands were broader as shown previously (Brumfeld et al., 1991). In our study, the correction has been made using the cholesterol spectrum from the KBr pellet since it is almost identical to the one obtained in benzene; it is assumed that organic solution provides an environment similar to the hydrophobic core of the lipid bilayer. Three cholesterol bands have been used as internal standards for the subtraction. The cholesterol bands at 1,388 and 1,365 cm^{-1} show up in the spectrum as shoulders on the 1,380 cm^{-1} band assigned to the methyl symmetric bending mode of the phospholipid (Casal and McElhaney, 1990). In addition, there is a weak cholesterol band at 1,333 cm^{-1} where the phospholipid does not contribute. The subtraction was made in such a way that these three bands were minimized, and the results obtained with these three check points were reasonable (see Fig. 1 *D*). The same correction factor has been used in the C—H stretching region. Despite a careful correction, the contribution of cholesterol does interfere in the methylene wagging region, and the quantitative band analysis in this region is practically impossible.

The effect of cholesterol on POPC as studied by infrared spectroscopy has not been reported yet. We briefly present our study, and compare the results with those on the well documented DPPC/cholesterol system (Umemura et al., 1980). The centers of gravity of the symmetric and antisymmetric C—H stretching bands as well as their widths have been measured for POPC and POPC/cholesterol mixtures in the fluid phase (T_m of POPC $\approx$ −5°C; De Kruyff et al., 1973). These measurements have been made after correction for cholesterol contribution. For the highest cholesterol content in the set of experiments (45 (mol) %), this correction shifts the symmetric and antisymmetric C—H stretching bands by 0.13 and −0.82 cm^{-1}, respectively. This shift is in the same order as that determined for a similar amount of cholesterol in phosphatidylserine (Brumfeld et al., 1991). The center of gravity of symmetric and antisymmetric CH_2 stretching bands versus cholesterol composition is plotted for various temperatures (Fig. 2). It can be seen that, at a given temperature, an increase in cholesterol content leads to a decrease of the center of gravity whereas, for a given cholesterol composition, the increase in temperature shifts the center of gravity towards high frequencies. Similar results have already been observed for other phospholipid systems (Umemura et al., 1980). It has been established that the shift of the these bands to higher frequencies indicates an increased disorder of the acyl chains (Asher and Levin, 1977).

Furthermore, it can be noted that cholesterol content does not produce a linear shift of the bands. The frequency decrease is steep between 10–30 (mol) % cholesterol, while the change is less pronounced for higher cholesterol content. Also, at low temperatures (0 and 10°C), the frequency slightly dips to a minimum at 30 (mol) % cholesterol.

Fig. 3 shows the width of symmetric and antisymmetric stretching bands of methylene groups for various cholesterol/lipid compositions at different temperatures. As can be seen, the widths follow a complex pattern. This could be due to the presence of more than one phase whose different band widths respond in a dissimilar fashion. A similar observation was made in the case of DMPC and glycophorin mixtures (Dluhy et al., 1983).

POPC-d_{31}/cholesterol system has been investigated by ^{2}H NMR (Lafleur et al., 1990b). In the conditions used in this study ($T \geq 0$°C, [cholesterol] $\leq$ 45 (mol) %), all the ^{2}H NMR spectra show typical powder patterns. Therefore, despite the large quantity of cholesterol

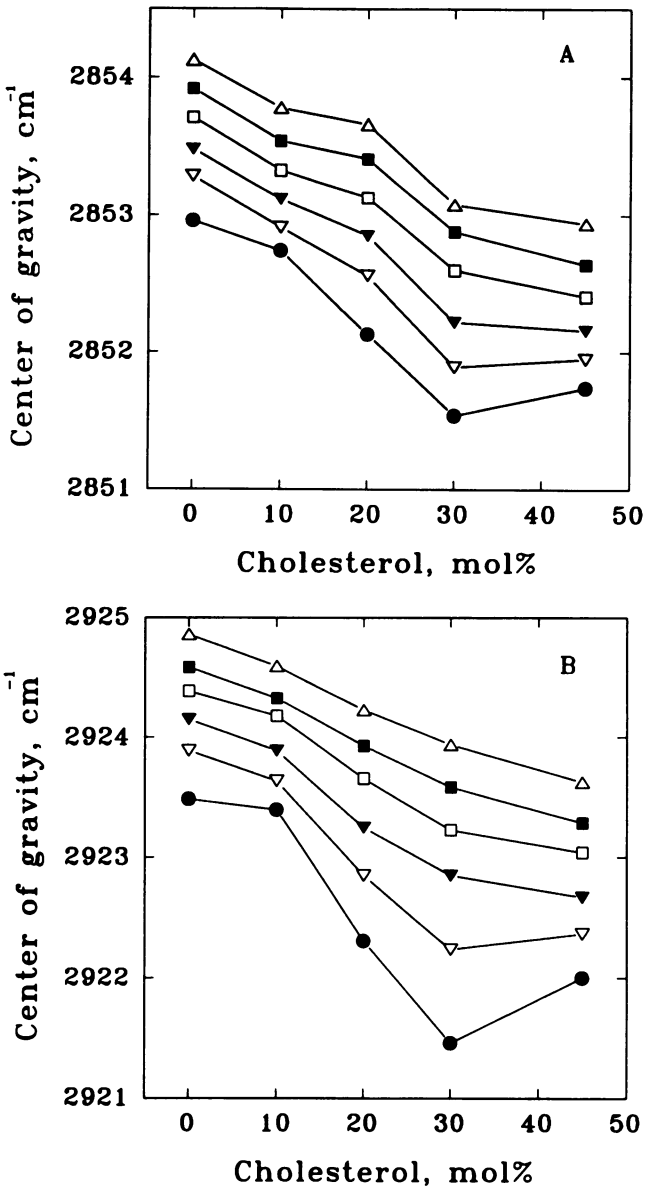

FIGURE 2 Effect of cholesterol on the centers of gravity of CH_2 (*A*) symmetric and (*B*) antisymmetric stretching bands of POPC in POPC/cholesterol mixtures at various temperatures: (●) 0°C, (▽) 10°C, (▼) 20°C, (□) 30°C, (■) 40°C, and (△) 50°C.

in some samples, the spectra indicate that the lipid phase remains fluid enough to allow fast axially symmetric reorientation of the lipids in the bilayers. The results are presented here in a convenient format for our purpose (Fig. 4); the mean orientational order parameter calculated over the whole perdeuteriated palmitoyl chain of POPC-d_{31} is plotted against cholesterol content for various temperatures. The mean orientational order parameters are increasing monotonically with increased cholesterol content or with decreased temperature. These trends in the effects of cholesterol on the NMR parameter have been observed for several lipid systems (Bloom et al., 1991). Fig. 4 also shows that the effect of cholesterol on $\langle S \rangle$ is not linear. The largest effect is found between 15–30 (mol) % cholesterol. The magnitude of the change is reduced for larger amount of cholesterol, and this reduction is more pronounced at low temperature. This behavior is similar to the one observed with the ν_{C-H} frequencies (Fig. 2).

Since temperature and cholesterol affect in a similar fashion the order parameter as measured by ^2H NMR and the band positions of the C—H stretching modes as measured by FTIR, we have plotted these parameters for different temperatures and cholesterol contents (Fig. 5). Surprisingly, a linear correlation is found between S and (*A*) the symmetric or (*B*) the antisymmetric ν_{C-H} fre-

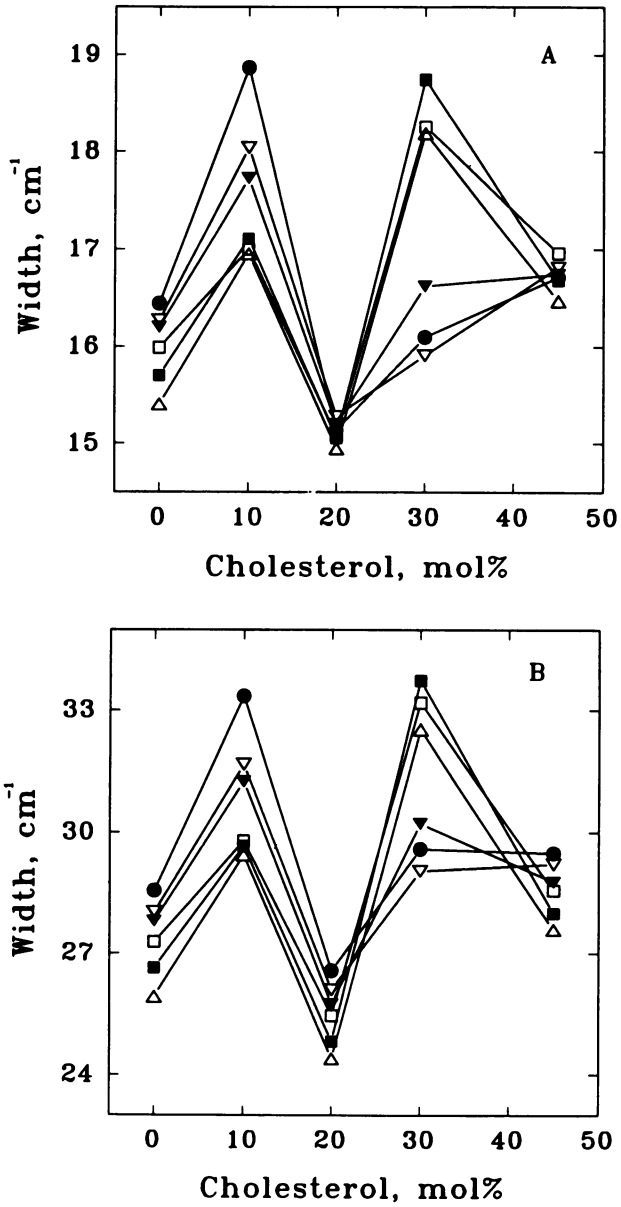

FIGURE 3 Effect of cholesterol on the width of CH_2 (*A*) symmetric and (*B*) antisymmetric stretching bands of POPC in POPC/cholesterol mixtures at various temperatures: (●) 0°C, (▽) 10°C, (▼) 20°C, (□) 30°C, (■) 40°C, and (△) 50°C.

quency. In addition, similar measurements have been made on 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE), and these data points are also included on the plot. The different systems are representative of a wide range of order, and the correlation appears to be valid over the whole domain investigated. The correlation coefficient and χ^2 for the fit between $\langle S \rangle$ and the symmetric stretching frequency is 0.98 and 0.00019, respectively, and those for the fit between $\langle S \rangle$ and the antisymmetric stretching frequency are 0.96 and 0.00031. As can be seen, the correlation is better with the symmetric stretching band, for which the cholesterol induced shift is smaller compared to that of antisymmetric stretching band.

DISCUSSION

It is established that ^{2}H NMR and IR measurements can be affected by several motions. On one hand, the orientational order is sensitive to molecular motions which cause the averaging of the orientation-dependent quadrupolar interactions; are included *trans/gauche* isomerisations and changes of the lipid director axis orientation caused by tilting of the molecules or by surface undulations (Davis, 1983; Meier et al., 1986). No distinction can be made among all the possible motions that are fast on the ^{2}H NMR time scale ($\approx 10^{-5}$ s). Several models have assumed that the quadrupolar splitting is mainly influenced by segmental reorientations due to *trans/gauche* isomerisation, and that rigid-body motions can be neglected (Seelig and Seelig, 1974; Meraldi and Schlitter, 1981; Salmon et al., 1987). Alternative approaches have included overall angular fluctuations of the acyl chains (Meier et al., 1986). At this point, no general conclusion has been established on the contribution of the different motions in the variation of S. On the other hand, the frequency of the C—H stretching bands is empirically related to *trans/gauche* isomerisation. However, it has been shown that there is a weak Fermi resonance interaction between the symmetric methylene stretching and binary combination modes involving the methylene bending vibrations (Snyder et al., 1978); so other phenomena such as lipid packing may indirectly

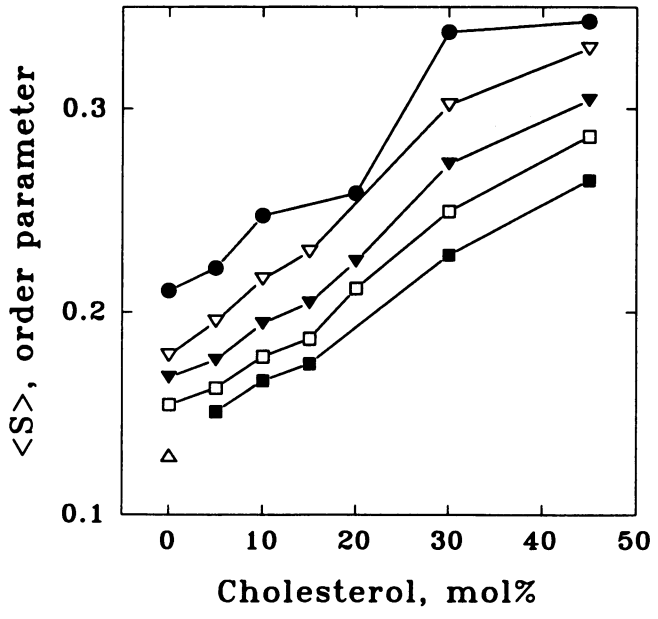

FIGURE 4 Effect of cholesterol on the mean orientational order parameter, $\langle S \rangle$, in POPC-d_{31}/cholesterol mixtures at various temperatures: (●) 0°C, (▽) 10°C, (▼) 20°C, (□) 30°C, (■) 40°C, and (△) 50°C.

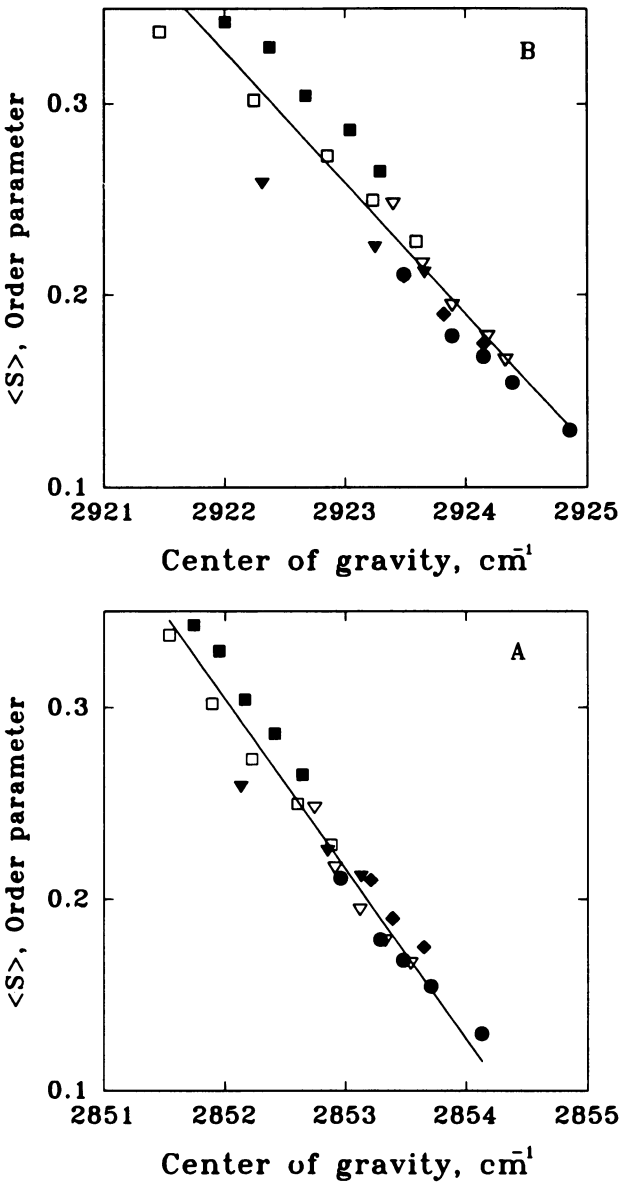

FIGURE 5 Plot of the mean orientational order parameter, $\langle S \rangle$, versus the center of gravity of (*A*) symmetric and (*B*) antisymmetric CH_2 stretching bands of the phospholipid in POPC/cholesterol mixtures and POPE at various temperatures. POPC/cholesterol mixtures; (●) 0, (▽) 10, (▼) 20, (□) 30, and (■) 45 (mol) % cholesterol. (♦) Pure POPE.

affect the C—H stretching region in IR spectroscopy, similar to what is observed in Raman spectroscopy.

The results presented here provide some insights into the origin of these measurements. The correlation observed between $\langle S \rangle$ measured by ^{2}H NMR and ν_{C-H} measured by infrared spectroscopy reveals that *both* parameters are chiefly sensitive to a common phenomenon in fluid bilayers. Amid all the motions mentioned above, *trans/gauche* isomerisation offers the most convincing explanation. These conclusions support the general understanding of orientational and conformational orders. It cannot be ruled out that the parameters are affected by more than one motion, but it is really improbable that two distinct motions affect in an equivalent manner the two parameters in such a way that the correlation can still be observed, especially when the nature of the three factors modulating the lipid chain order is different. Therefore, the correlation presented here constitutes an experimental indication that, to a first approximation, the variation of orientational and IR orders in the fluid phase is primarily dictated by *trans/gauche* isomerisation, and that other motions play a minor role. It should be noted that the linear fit obtained between $\langle S \rangle$ and ν_{C-H} does not imply that each parameter varies necessarily in a linear manner with the number of gauche bonds in the chains. Actually, it has been shown that the relation between S and the number of gauche bonds is not straightforward since S depends on the orientation of the C—D bond relative to the bilayer normal (Seelig and Seelig, 1974). We would like also to stress the fact that the correlation presented here has been observed for phospholipids bearing a sn1 palmitoyl and sn2 oleoyl chains. S and ν_{C-H} can be affected by several chain characteristics including the location of the deuterium nuclei (Seelig and Seelig, 1980), the presence of unsaturation (Salmon et al., 1987) and the chain length (Umemura et al., 1981). Therefore, the extrapolation to phospholipids with different chains is not straightforward.

Time consideration

It is essential to consider the time scale of these measurements. This question has been addressed previously (Bloom et al., 1991), and it is clear that ^{2}H NMR and infrared spectroscopies do not respond on the same time scale. For the NMR spectroscopy, the quadrupolar interactions give rise to a signal spread over $\approx$100 kHz; the time scale associated with the measurement is approximately 10^{-5} s. So, the quadrupolar interactions are averaged by *trans/gauche* isomerisation since the lifetime of a conformer is evaluated to be 10^{-10} s. The measured quadrupolar splitting is related to *the time-averaged value* of S.

Conversely, infrared spectroscopy shows well resolved bands assigned to the different conformers as can be clearly observed for the CH_2 wagging (Snyder, 1967) and the CD_2 rocking (Snyder and Poore, 1973) modes of the methylene groups. This is a consequence of the characteristic frequency associated with the vibrational spectroscopy which is greater than the rate of *trans/gauche* isomerisation. For the ν_{C-H} modes however, these distinct signals cannot be resolved because the magnitude of the effect of conformational disorder on the ν_{C-H} frequency is relatively small, as the C—H bond is not directly involved in the isomerisation. However, it is very likely that the ν_{C-H} is a *combination* of different signals assigned to different conformers.

Spatial consideration

For the ^{2}H NMR measurement, the value of $\langle S \rangle$ corresponds to *the mean value* of $S(n)$ calculated over the palmitoyl chain for $2 \leq n \leq 16$, with an equal weight for each position. For the IR measurement, the bands observed for the methylene ν_{C-H} do not correspond to a single species, and three main contributions must be taken into account. First, there is a contribution from the methylene groups of both the sn1 and sn2 acyl chains; it has been shown by Raman spectroscopy and ^{2}H NMR that these two chains are not equivalent (Gaber et al., 1978; Seelig and Seelig, 1980). Second, the C—H stretching modes of methylene group are sensitive to the position of the methylene group along the chain. It has been shown by Raman spectroscopy that, in the case of palmitic acid at liquid nitrogen temperature, the antisymmetric stretching of the CH_2 group in α position relative to the carboxylic group appears at 2,925 cm^{-1} while this mode is observed at 2,899 cm^{-1} for the methylene adjacent to the terminal methyl (Hill and Levin, 1979). A similar change has been observed in the Raman spectrum of specifically deuteriated dimyristoylphosphatidylcholine in the gel phase. The symmetric C—D stretching frequency varies from 2,114 cm^{-1} for the CD_2 group next to the ester link, to 2,094 cm^{-1} for the CD_2 group at the 12th position (Bansil et al., 1980). This effect levels off after the 6th methylene group. The difference of $\sim$20 cm^{-1} existing along ordered chains has been attributed to the influence of the charge distribution of the polar ester group (Bansil et al., 1980). And third, for a given position of a given chain, the different conformers also contribute to the C—H stretching region. This latter contribution is the crucial one in our study. Indeed, the ν_{C-H} bands reflect the probability distribution of the three contributions over all the lipid chains, and a detailed analysis taking into account the various contributions is extremely difficult. However, the measurement of the center of gravity of these bands describes this complex distribution, providing *the most probable* frequencies of the C—H stretchings.

General consideration

Since ^{2}H NMR and IR measurements do not respond on the same time scale, do not probe the very same portions of the acyl chains, and do not reflect in the same way the order distribution (mean versus most probable value), it

is surprising to observe such a good correlation between them. A recent ^2H NMR study has proposed that the order distribution along the lipid acyl chains behaves in a first approximation in a universal manner (Lafleur et al., 1990b). This conclusion has been extended to different chain lengths, if position along the chain is normalized (Morrow and Lu, 1991). This universal behavior of the order in the hydrophobic core of fluid bilayer indicates that the order depends on only few parameters; this phenomenon is very likely at the origin of the correlation between the average orientational order parameter and the most probable frequency of the C—H stretchings. Actually, at this point, there is a significant number of interrelated parameters describing the acyl chain order. As mentioned previously, there is a general correlation between $\langle S \rangle$ and the bilayer thickness or the molecular area (Seelig and Seelig, 1974; Ipsen et al., 1990). The orientational order has been also related to the lateral pressure existing in the apolar core (Meraldi and Schlitter, 1981). These correlations and the one presented in this paper indicate that several measurements related to the hydrophobic core of the bilayer reveal different aspects of a more general behavior, and they should give a unified view of the bilayer. It would be of no surprise if future investigations reveal similar correlations. In addition, we believe that research bridging the different measurements should be encouraged.

The correlation obtained between NMR and IR data has also been investigated for gel phase DPPC. It is known that, in the gel phase, several molecular motions are slowed down, and this variation induces important changes in the NMR band shape (Vist and Davis, 1990). In the gel phase, lipids do not show axial symmetry on the NMR time scale. Hence, we have done the comparison between the first moment of the ^2H NMR spectra of DPPC-d_{62} and the center of gravity of the methylene C—H stretching bands (data not shown). In this case, no linear correlation has been found; the relation found in the fluid phase cannot be extended to the gel phase. The slower motions affecting the NMR lineshape (so the first moment) should not have an important effect on the infrared parameters, and are possibly at the origin of the observed deviation. An interesting observation is worth noting: when cholesterol content in the sample is increased to 20 (mol) %, the data points obtained at low temperatures ($T \leq 30°C$) get closer to the straight line extrapolated from the values obtained for lipids in the liquid crystalline phase ($T \geq 40°C$). We interpret this as a direct consequence of the formation of the liquid ordered phase (Vist and Davis, 1990) leading to rapid axially symmetric reorientation on the NMR time scale.

At the outset of this work, we also wanted to compare the ^2H NMR results with the conformational order obtained from the CH_2 wagging region. It turned out that the interference from cholesterol bands was, in our hands, impossible to correct in such a way that reproducible and quantitative results can be obtained. So, in this respect, the prediction of spectral interferences from additive membrane components (Senak et al., 1991) was correct. Also, the width of the ν_{C-H} bands show a complex behavior. The width of an infrared band is sensitive to the perturbation of neighboring molecules (Ramsay, 1952; Rothschild, 1976), and is probably not related to *trans/gauche* isomerisation in a simple manner.

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