Kirk, G. L., Gruner, S. M., & Stein, D. L. (1984) *Biochemistry 23*, 1093-1102.
Knoll, W., Schmidt, G., Ibel, K., & Sackmann, E. (1985) *Biochemistry 24*, 5240-5246.
Laggner, P. (1984) in *Structural Biological Applications of X-ray Absorption, Scattering and Diffraction* (Chance, B., & Bartunik, H. D., Eds.) pp 171-182, Academic Press, London.
Murari, R., Murari, M. P., & Baumann, W. J. (1986) *Biochemistry 25*, 1062-1067.
Navarro, J., Toivio-Kinnucon, M., & Racker, E. (1984) *Biochemistry 23*, 130-135.
Reich, M. K., Kam, Z., & Eisenberg, H. (1982) *Biochemistry 21*, 5189-5195.
Seddon, J. M., Cevc, G., & Marsh, D. (1983) *Biochemistry 22*, 1280-1289.
Shipley, G. G. (1973) in *Biological Membranes* (Chapman, D., & Wallach, D. F. M., Eds.) Vol. 2, pp 1-89, Academic Press, London.
Valtersson, C., van Duyn, G., Verkleij, A. J., Chojnachi, T., De Kruijff, B., & Dallner, G. (1985) *J. Biol. Chem. 260*, 2742-2751.
Verkleij, A. J. (1984) *Biochim. Biophys. Acta 779*, 43-63.
Wachtel, E., & Bach, D. (1987) *Biochim. Biophys. Acta 922*, 234-238.
Wieslander, A., Rilfors, L., & Lindblom, G. (1986) *Biochemistry 25*, 7511-7517.
Yeagle, P. L. (1985) *Biochim. Biophys. Acta 822*, 267-287.

Quantitative Determination of Conformational Disorder in the Acyl Chains of Phospholipid Bilayers by Infrared Spectroscopy†

R. Mendelsohn,*,‡ M. A. Davies,‡ J. W. Brauner,‡ H. F. Schuster,‡ and R. A. Dluhy*,§

Department of Chemistry, Rutgers University, 73 Warren Street, Newark, New Jersey 07102, and National Center for Biomedical Infrared Spectroscopy, Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201

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ABSTRACT: A method is proposed and demonstrated for the direct determination of conformational disorder (trans-gauche isomerization) as a function of acyl-chain position in phospholipid bilayer membranes. Three specifically deuterated derivatives of dipalmitoylphosphatidylcholine (DPPC), namely 4,4,4′,4′-d_4-DPPC (4-d_4-DPPC), 6,6,6′,6′-d_4-DPPC (6-d_4-DPPC), and 10,10,10′, 10′-d_4-DPPC (10-d_4-DPPC), have been synthesized. The CD_2 rocking modes in the Fourier transform infrared (FT-IR) spectrum have been monitored as a function of temperature for each derivative. A method originally applied by Snyder and Poore [(1973) *Macromolecules 6*, 708-715] as a specific probe of hydrocarbon chain conformation in alkanes has been used to analyze the data. The rocking modes appear at 622 cm^{-1} for a CD_2 segment surrounded by a trans C-C-C skeleton and between 645 and 655 cm^{-1} for segments surrounded by particular gauche conformers. The integrated band intensities of these modes have been used to monitor trans-gauche isomerization in the acyl chains at particular depths in the bilayer. At 48 °C, above the gel-liquid-crystal phase transition, the percentage of gauche rotamers present is 20.7 ± 4.2, 32.3 ± 2.3, and 19.7 ± 0.8 for 4-d_4-DPPC, 6-d_4-DPPC, and 10-d_4-DPPC, respectively. The gel phase of the latter two molecules is highly ordered. In contrast, a substantial population of gauche rotamers was observed for the 4-d_4-DPPC. The conformational analysis yields a range of 3.6-4.2 gauche rotamers/acyl chain of DPPC above the phase transition. This range is in excellent accord with the dilatometric data of Nagle and Wilkinson [(1978) *Biophys. J. 23*, 159-175]. The significant advantages of the FT-IR approach are discussed.

Determination of the structure and dynamics of phospholipids, and the relationship of these quantities to the function of membrane proteins, is a long-sought goal of membrane biophysics. Toward this end, the arsenal of modern spectroscopic technology has been brought to bear. Each technique contributes information on its own time scale to a composite picture of phospholipid organization. Yet a quantitative picture of the contribution of any single motion to phospholipid dynamics is elusive. The reason for this is not difficult to understand. For example, the most powerful technique brought to bear on this problem to date is probably ^2H NMR spectroscopy [for a recent review, see Seelig and MacDonald (1987)]. The order parameters derived from this experiment incorporate all motions faster than about 10^{-5}-10^{-6} s, the characteristic time scale for the NMR measurement (Seelig & Seelig, 1974; Petersen & Chan, 1977). Thus, the contribution from any one of the possible motions (trans-gauche isomerization, acyl-chain librations, rigid body motions, etc.) to the spectrum is difficult to determine. Other commonly used spectroscopies, primarily fluorescence and ESR, are exquisitely sensitive, but in general require the use of probe molecules. These at best present difficulties in transferring the measured spectral properties of the probe to the physical properties of the phospholipid and at worst have the potential to perturb the order and dynamics of the system under investigation (Taylor & Smith, 1980).

Infrared and Raman spectroscopies, in principle, operate on a time scale that has the potential to sample directly the

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* Address correspondence to these authors.
‡ Rutgers University.
§ Battelle Memorial Institute.

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fastest conformational alterations that occur in biological membranes, namely acyl-chain trans–gauche isomerizations. Quantitative determination of this motion is desirable both for the calibration of theoretical models for membrane structure and for data interpretation from other spectral techniques where trans–gauche isomerization forms but one component of the detected motion.

To date, vibrational spectroscopy has been used primarily in a qualitative way to monitor lipid-phase changes and the effect of other membrane components on them. Several widely used indices of acyl-chain order are extant—e.g., the 2850-cm^{-1} frequency of the acyl-chain C–H stretching modes (Mendelsohn & Mantsch, 1986) from FT-IR spectra, the 2935/2880 and 2850/2880 intensity ratios from phospholipid Raman spectra (Mushayakarara et al., 1982; Taraschi & Mendelsohn, 1980), and the intensity of the 1130-cm^{-1} band arising from C–C stretching frequencies of the acyl chains in the trans conformation (Gaber & Peticolas, 1977; Yellin & Levin, 1977; Lieb et al., 1982; Levin & Bush, 1982).

Yet it has been difficult to correlate these empirical spectral parameters with a quantitative description of trans–gauche isomerization in the acyl chains. Several models have appeared for the intensity of the 1130 cm^{-1} band in this context (Gaber & Peticolas, 1977; Pink et al., 1980). These, in turn, have been criticized on the grounds that the mode is sensitive not only to trans–gauche isomerization but also to additional structural reorganizations in bilayers (Snyder et al., 1980).

The current study describes the successful measurement of the CD_2 rocking modes in the IR spectra of specifically deuterated phospholipids and their implementation as direct and quantitative probes of conformational order in phospholipids that have been deuterated at specific acyl-chain positions. Integrated band intensities have been incorporated in a quantitative model for the fraction of gauche rotamers formed at particular positions (depths) in the bilayer. The method was originally proposed by Snyder and Poore (1973) as a specific probe of hydrocarbon chain conformation in alkanes and later applied by Snyder, Strauss, and their co-workers (Maroncelli et al., 1985a,b; Shannon et al., 1989) to monitor the distribution of gauche bonds in a variety of solid (rotator) phases in these systems. The current application of the method is the first to the study of conformational disorder in phospholipid bilayers.

Materials and Methods

Syntheses of Specifically Deuterated Phospholipids. Syntheses of 4,4,4′,4′-d_4-dipalmitoylphosphatidylcholine (4-d_4-DPPC), 6,6,6′,6′-d_4-dipalmitoylphosphatidylcholine (6-d_4-DPPC), and 10,10,10′,10′-d_4-dipalmitoylphosphatidylcholine (10-d_4-DPPC) were modifications (H. F. Schuster, unpublished results) of the procedures of Tulloch (1979) scaled to yield 2–3 g of each phospholipid. The various derivatives were fully characterized by NMR, mass spectrometry, and FT-IR. Differential scanning calorimetry scans for each derivative displayed a sharp (half-width 0.3 °C) main transition at 41 °C and a pretransition at about 32 °C.

Sample Preparation. Complete sample hydration is critical to the success of this experiment. Incomplete hydration leads to the appearance of extra FT-IR feautres of unknown origin throughout the CD_2 rocking region (600–660 cm^{-1}), vitiating the results. Such spurious bands were observed repeatedly in numerous attempts to measure the acyl-chain isomerization process by use of attenuated total reflectance (ATR) techniques. Furthermore, ATR measurements were rendered more difficult by the appearance of interference fringes of greater intensity than the sought spectral features.

Conditions for observations of CD_2 rocking modes were optimized in a transmission experiment. Ten milligrams of specifically deuterated lipid and 20 μL of D_2O were added to a culture tube, which was then sealed. The mixture was held at 55 °C, with periodic agitation, for at least 1 h, and usually longer, to ensure complete hydration. Hydrated samples were placed between two AgCl windows, contained and sealed with a 6-μm spacer. The circumference of this assembly was wrapped with Teflon tape, forming a further seal against dehydration. The assembly was then inserted into a variable-temperature Harrick cell. Temperature control was achieved with a Haake circulating water bath and monitored with a thermocouple placed adjacent to the point where the IR radiation was focused. Temperatures are estimated accurate to ±1 °C.

FT-IR Data Acquisition. Spectra (Rutgers University) were acquired with a Mattson Instruments Sirius 100 FT-IR spectrometer (Mattson Instruments, Madison, WI) equipped with a HgCdTe detector (1 × 1 mm) fabricated (Infrared Associates, Cranbury, NJ) to give high sensitivity near 620 cm^{-1}; 2000 scans of sample and background were co-added and apodized with a triangular function. Spectrometer resolution was 4 cm^{-1}. Interferograms were zero-filled (two levels) and fast Fourier transformed to yield data encoded every 1 cm^{-1}. Band positions, determined with a center of gravity algorithm (Cameron et al., 1982), had an uncertainty of less than ±0.1 cm^{-1}.

A parallel series of spectra for each of the specifically deuterated phospholipids were obtained on a Digilab FTS-40 FT-IR spectrometer (Digilab, Inc., Cambridge, MA) located at the Battelle laboratories. These IR spectra were collected by co-addition of 1024 interferograms at 2-cm^{-1} resolution with one level of zero-filling and apodized with a triangular function to give a final data encoding interval of 1 cm^{-1}. For these experiments, the spectrometer was equipped with a wide-band (~400 cm^{-1} cutoff) HgCdTe detector.

FT-IR Data Reduction. The CD_2 rocking modes discussed in this study appear between 600 and 660 cm^{-1} as very weak features on a large D_2O libration background. Typically this sloping background is 0.8–1.2 absorbance units under the experimental conditions described above. Background subtraction is necessary because the strongest band of this spectral region is about 30 milliabsorbance units in the liquid-crystal phase. Fully proteated DPPC, hydrated with molar proportions of D_2O identical with those of the deuterated sample and measured under the same conditions of cell path length and temperature, is used as the background for subtraction. Subtraction is completed when minima between 610 and 660 cm^{-1} have equal absorbance values.

Incomplete resolution of spectral features made curve fitting for quantitative analysis of band intensities necessary. Band profiles constructed from addition of individual Gaussian–Lorentzian bands were compared with experimental spectra by using an interactive algorithm written specifically for this purpose (J. Brauner, unpublished results). Areas of the component bands were computed and used for calculating the relative intensities of various conformers.

An alternative method of background subtraction was used at the Battelle laboratories to visualize the weak CD_2 rocking modes in the region 600–700 cm^{-1}. In this method, a multipoint polynomial function was fit to the sloping background in the region between 685 and 605 cm^{-1}. This function was then subtracted from the spectrum of the deuterated lipid to give a flat base line in this region. Curve fitting of the resulting spectrum to obtain quantitative band intensities and areas was

Table I: Percent of Total[a] Gauche Conformers for Specifically Deuterated DPPC Derivatives

	temp (°C)			
derivative	20	33 ± 1	38	48.5 ± 0.5
4-d_4-DPPC	7.6	10.9 ± 6.1 (4)[b]	11.1 ± 6.8 (4)	20.7 ± 4.2 (5)
6-d_4-DPPC		1.7 ± 0.3 (4)	2.3 ± 1.1 (3)	32.3 ± 2.3 (4)
10-d_4-DPPC		2.5		19.7 ± 0.8 (2)

[a] The totals neglect gg conformers. See text. [b] Number of measurements in parentheses.

Table II: Percent of tgt[a] and tgg[a] Conformer Classes for Specifically Deuterated DPPC Derivatives at 48 °C

derivative	tgt class (%)	tgg class[b] (%)
4-d_4-DPPC	84.6 ± 8.9 (5)	15.4 ± 8.9
6-d_4-DPPC	74.4 ± 18.1 (5)	25.6 ± 18.1
10-d_4-DPPC	85.9 ± 8.0 (2)	14.1 ± 8.0

[a] Conformer classes are defined in the text. [b] Determined by difference.

accomplished by using a least-squares algorithm (Fraser & Suzuki, 1973). The areas and intensities of the calculated component bands were used in the calculation of the relative conformer percentages. The data given in Tables I and II were calculated by using spectra recorded both at the Battelle laboratories and at Rutgers University. Good agreement was obtained between the two methods described above for background subtraction, as reflected by the standard deviations reported in Table I, which are based on all the data obtained in both laboratories.

Theory

Maroncelli et al. (1985a,b) have shown that the frequency of a CD_2 rocking mode substituted into a hydrocarbon chain is sensitive to conformation in the immediate neighborhood of the CD_2 moiety. We follow their suggestion and italicize the C–C bonds that are directly bonded to the CD_2. Thus, *tg* means the CD_2 group under study adjoins a trans (t) and a gauche (g) bond. The CD_2 probe frequency is dependent on the C–C bond or bonds neighboring the *tt* and *tg* bond pairs. In particular, there are three discrete frequencies in the 620-660-cm^{-1} spectral region that are relevant to the current work. An IR-active band occurs at about 622 cm^{-1} if the CD_2 group adjoins two trans bonds, independent of the conformation of neighboring C–C bonds. This class of conformers will be termed the tt class. A second band at about 652 cm^{-1} consists of conformations of the type g′*g*t (kink) and t*t*g*t* (single gauche bend). The final band of interest for conformational study appears at 646 cm^{-1} and is assigned as the CD_2 rocking modes arising from t*t*g*g* or g′*t*g*g*. Maroncelli et al. (1985a,b) have shown that the rocking frequencies are not sensitive to the conformation of C–C bonds more than two bonds away from the CD_2 group. Conformers of the class giving rise to the band at 652 cm^{-1} are designated *tg*t, and those of the class giving rise to the 646-cm^{-1} feature are designated *tg*g. Taken together, these two classes delineate the extent of disorder at a given chain position. In all current experiments, the 646-cm^{-1} band is much weaker than the 652-cm^{-1} band as expected from the nature of the conformers. The *gg* conformations themselves are expected to have a weak broad band calculated at 680 cm^{-1} but observed in cycloalkanes (Shannon et al., 1989) at 665 cm^{-1}. These rare conformers are neglected in the current analysis.

As trans–gauche isomerization occurs on a time scale slower than the vibrational period, the various conformations give rise to discrete (albeit weak) bands in the IR. The ratio of gauche (n_g) to trans (n_t) forms is given by

$$\frac{n_g}{n_t} = \frac{I(652) + I(646)}{(I(622))A} = I_{exp} \quad (1)$$

where the factor A allows for differences in unit absorptivity between the bands and I_{exp} is the experimentally measured intensity ratio.

The factor A was determined (Maroncelli et al., 1985) from a statistical model of alkanes and a calculation of the band intensities from the square of the dipole moment derivatives for the particular normal modes. An energy difference of (508 ± 50) cal/mol was assumed for the relative energy of *g* vs *t*. This led to $A = 1.00 \pm 0.07$, the uncertainty reflecting the range of experimental values for the energy difference. The individual bands of the 652/646-cm^{-1} doublet are also calculated to have equal intensities; thus, no further corrections to the numerator of eq 1 are required. The fraction of gauche rotamers is thus computed from

$$\frac{n_g}{n_g + n_t} = \frac{1}{1 + (1/I_{exp})} \quad (2)$$

Results

The spectral regions of interest in the current work encompass the CD_2 stretching region (2000–2300 cm^{-1}) and the CD_2 rocking modes (600–660 cm^{-1}). Typical data above and below the gel–liquid-crystal phase transition (41 °C) for these spectral regions, respectively, are shown in part A and B of Figure 1 for 6-d_4-DPPC. These spectra have had solvent background subtracted and have been flattened as described under Materials and Methods. The symmetric and asymmetric CD_2 stretching frequencies near 2100 and 2200 cm^{-1}, respectively, have been used as qualitative probes of the gel–liquid-crystal phase transition by Raman spectroscopy (Bansil et al., 1980) and by IR spectroscopy (Cameron et al., 1981). To demonstrate that the current set of DPPC derivatives have thermotropic behavior resembling the fully proteated molecule, the frequency of the symmetric CD_2 stretching mode has been monitored as a function of temperature. The results are shown in Figure 2. The cooperative transition with T_m at 41 °C is in good accord with the well-known gel → liquid-crystal transition temperature for this molecule (Hinz & Sturtevant, 1972). Thus, it is evident that insertion of four deuterons does not significantly alter the thermotropic behavior of the molecule and that hydration of the sample is complete under the current experimental conditions. Incomplete hydration is known to significantly increase T_m (Chapman, 1975).

The conformationally sensitive CD_2 rocking modes for 6-d_4-DPPC above and below T_m are shown in Figure 1B. The bands are rather weak in intensity—the absorbance values (not shown in the figure) range from 0.040 for the 622-cm^{-1} trans band in the gel phase to less than 0.001 for the 652- or 646-cm^{-1} gauche bands in the gel phase. The noise level after data reduction is estimated at 0.0001 unit. The complex contours in this spectral region have been decomposed as discussed under Materials and Methods, and the resultant fractions of total gauche (*tg*t + *tg*g classes) conformers derived from the intensities of the component bands for the 4, 6, and 10-d_4-DPPC derivatives studied are listed in Table I. An example of the quality of the fit is given in Figure 3 for 6-d_4-DPPC above T_m. The spectral assignments for the bands at about 622, 646, and 652 cm^{-1} have been discussed above. Additional weak features in the 610–630-cm^{-1} region are of unknown origin, but they contribute less than 3% of the intensity of the main 622-cm^{-1} band and thus do not seriously affect the conformational analysis below. A band near 661 cm^{-1} is relatively insensitive to conformational alterations and has been

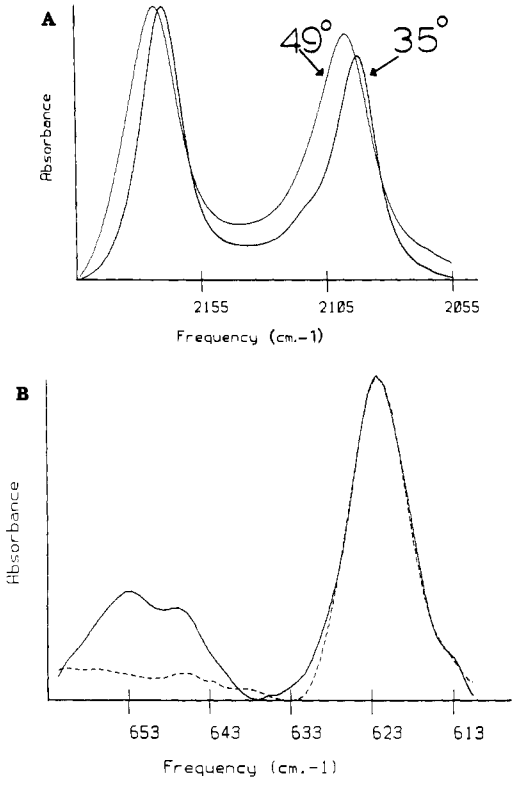

FIGURE 1: (A) FT-IR spectra in the C–D (2055–2200 cm^{-1}) stretching region for 6-d_4-DPPC at the indicated temperatures above (49 °C) and below (35 °C) T_m. The frequency of the symmetric stretch near 2100 cm^{-1} is measured and used to construct a melting curve (Figure 2). (B) FT-IR spectra in the CD_2 rocking region (610–665 cm^{-1}) for 6-d_4-DPPC at 33 (---) and 48 °C (—). The bands near 646 and 653 cm^{-1} arise from gauche conformers as described in the text. The weak intensity in the gel phase implies a highly ordered system. The band near 622 cm^{-1} arises from trans conformers. These spectra have not been subjected to spectral smoothing algorithms and reflect the raw data after background subtraction and base-line flattening. The absorbance of the 622-cm^{-1} band at 48 °C is 18 milliabsorbance units above the background.

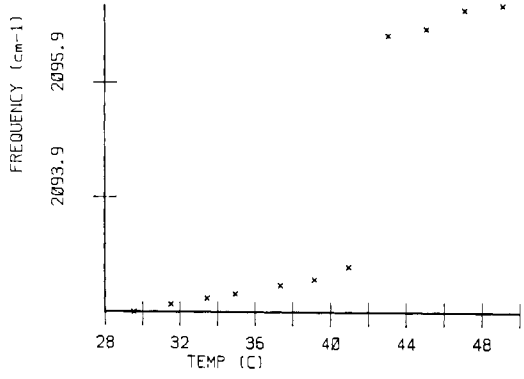

FIGURE 2: Melting curve for 6-d_4-DPPC constructed from the frequency of the CD_2 symmetric stretching mode. The sharp transition near 41–42 °C is the main phase transition.

assigned by Maroncelli et al. (1985) as arising from CHD rocking modes of incompletely deuterated methylenes. It is therefore irrelevant to the current analysis. The results are depicted in graphical form in Figure 4. In Table II, the relative contributions of the individual *tgt* (652 cm^{-1}) and *tgg* (646 cm^{-1}) bands to the liquid-crystlaline state of the various derivatives are summarized.

Discussion

As noted above, insertion of CD_2 groups at specific sites on the phospholipid acyl chains provides a set of nonperturbing

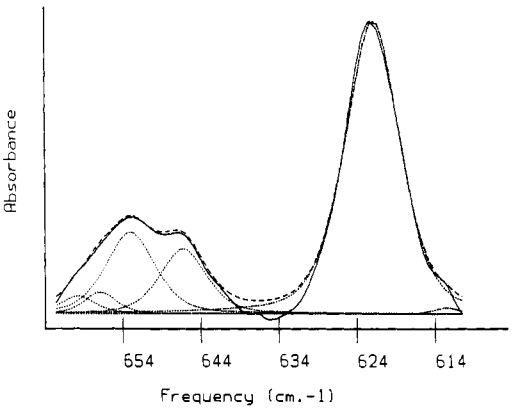

FIGURE 3: Results of a typical curve-fitting process for band decomposition of the CD_2 rocking region. The experimental spectrum is the solid line (—). The dotted curves (···) are the component bands. The dashed line (---) represents the sum of the component bands.

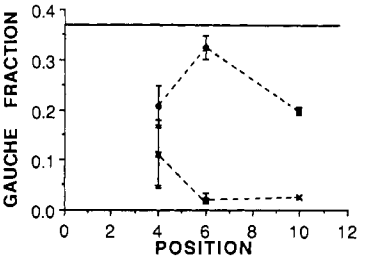

FIGURE 4: Graphical summary of results showing disordering of the DPPC acyl chains as a function of depth in the bilayer. The error bars for each data point are drawn in accord with the data in Table I. Temperatures are (○) 48.5, (△) 38, and (×) 33 °C. The horizontal line represents the theoretical maximum disorder for a random coil at 41 °C (Gruen, 1982).

IR structural probes. The current experiment allows the following issues to begin to be quantitatively addressed: (1) What are the fractions of trans and gauche conformers present in the gel and liquid-crystal phases? (2) What is the depth dependence of the trans/gauche population ratio along the acyl chains? (3) What types of conformers are present at each position? (4) How do the measurements of trans–gauche isomerization compare with the order parameters of ^2H NMR spectroscopy when the latter are analyzed to remove contributions from slower motions?

The assumptions inherent in the analysis of these data are as follows: (1) The relative gauche/trans absorptivities (factor *A* in eq 1) are the same in phospholipids as in alkanes. As the vibrational modes are highly localized (thus not sensitive to bulk phase structure), this assumption is well justified. (2) The vibrational bands are sensitive only to trans–gauche isomerization. The rapid time scale should "uncouple" these vibrational frequencies from the slower motions that appear in NMR.

Several different experimental approaches have been used to generate estimates of the extent of conformational order in the phospholipid acyl chains. Yellin and Levin (1977), from measurements of the C–C stretching bands near 1090 and 1130 cm^{-1} in the Raman spectrum, estimate the occurrence of one gauche bond/acyl chain in the gel phase of DPPC. Gaber and Peticolas (1977) used the same 1130-cm^{-1} feature to define an order parameter related to the number of trans bonds in the acyl chains. They conclude that 12 trans bonds exist/acyl chain in a dispersion of DPPC at 30 °C, two trans bonds are lost between 34 and 41 °C (e.g., between the pretransition and the main transition), and an additional five bonds are lost at the main transition. The basic assumption

of their analysis is that the intensity of the 1130-cm^{-1} mode is a constant per "trans-unit", independent of neighbor and end-group effects. Implicit also is the idea that the 1130-cm^{-1} intensity changes are derived solely from trans–gauche isomerization. The latter assumption was challenged by Snyder et al. (1980), who showed that in *n*-heneicosane (n-$C_{21}H_{44}$) the 1130-cm^{-1} band decreased by 20% on transition of this molecule from the orthorhombic to the hexagonal phase, a transition during which the all-trans conformation is preserved.

A different approach was proposed by Pink et al. (1980) in which the formalism of Theimer (1957) was used to calculate the 1130-cm^{-1} intensity for particular conformations. Practical application of the model requires a theory of chain conformations in which the locations of gauche conformations can be predicted. The model has been criticized by Vogel and Jahnig (1981). The quantitative applications of vibrational spectroscopy to date to the problem of conformational order in phospholipids are thus quite confused. The basic difficulty arises because the 1130-cm^{-1} mode has internal coordinates from any section of the molecule that is in the trans configuration. The presence of end effects and conformation of neighboring groups, as suggested by Gaber and Peticolas (1977), and the apparent sensitivity of the mode to structural changes not related to trans–gauche isomerization, create difficulty with the detailed analysis.

Other physical techniques have been applied to this problem. Nagle and Wilkinson (1978) measured the volume change at the main transition, estimated the energy difference accompanying this change, and deduced that the change in the probability of a gauche rotamer at the main transition [Δp_g in the notation of Nagle and Wilkinson (1978)] is 0.23. They have not dealt with the position dependence of this probability. Their analysis leads to an estimate of 3.8 gauche rotamers/chain above T_m (7.6/molecule) for DPPC.

Several estimates of intramolecular order in the acyl chains have been made on the basis of NMR measurements. Seelig and Seelig (1974), in their pioneering application of ^2H NMR, have analyzed the measured deuterium order parameters and recognized a plateau of constant values from position 2 to position 10 in the acyl chain of DPPC and decreased order from position 10 to the terminal methyl region. By selecting particularly likely conformations and analyzing their contributions to the order profiles, the Seeligs determined that the lower limit of gauche states per chain was three. To conform to the estimated energy difference of 500 cal/mol between t and g states, an upper limit of six gauche states per chain was estimated. Petersen and Chan (1977) have elaborated a theoretical model invoking chain reorientation and chain isomerization via kink diffusion to explain proton and deuterium order parameters. They calculate a probability of trans orientation of a methylene segment in the upper part of the chain to be about 0.8–0.9 in the liquid-crystalline state.

The current experimental determination of configurational order at particular chain positions can be directly compared with some of the above results. Our observation of 20.7 ± 4.2, 32.3 ± 2.3, and 19.7 ± 0.8% gauche rotamers at the 4, 6, and 10 positions, respectively, of DPPC at 48 °C is in fair agreement with Petersen and Chan's model. Within the limits of current experimental precision (Table I) there is insignificant difference in gauche rotamer probability between positions 4 and 10, although a real increase appears at the 6-position.

To estimate the number of gauche rotamers/chain requires multiplication by the appropriate number of C–C bonds, naturally excluding the C_{15}–C_{16} bond. This results in 3.6 gauche bonds/chain, assuming an average of our FT-IR measurements along the entire chain. It is noted this is probably a lower bound to the true number, as significantly more disorder is expected from C_{11}–C_{16}, an area of the bilayer not probed in the current work. The appropriate deuterated derivatives of DPPC are currently being synthesized to evaluate conformational order toward the bilayer center. Assuming a maximum value of 36.8% [for a random coil at 41 °C (Gruen, 1982)] for bonds 11–15 yields a maximum number of about 4.2 gauche bonds/chain. These results (lower limit = 3.6; upper limit = 4.2) are in excellent accord with the analysis of the dilatometric data by Nagle and Wilkinson (1978) and with the lower range of the estimate of the Seeligs (1974) from NMR measurements. Examination of Figure 4 confirms that significant restrictions to maximum disorder exist in the upper half of the chain compared to a random coil, for which, as noted above, 0.368 of the bonds are expected to be gauche.

Conformational order in the gel state at 33 °C is very high for the 6- and 10-positions of the acyl chains, where only 1.5–3% gauche bonds are found (Table I). The large fractional uncertainties (Table I) reflect the low absorbance values of the gauche marker band (<0.001 absorbance unit for the gauche doublet at 652/646 cm^{-1}). These data are in excellent agreement with those of Vogel and Jahnig (1981), who used the frequency of the longitudinal acoustical mode in the Raman spectrum to monitor the average all-trans segment length in dimyristoylphosphatidylcholine and in dihexadecylphosphatidic acid in their respective gel phases. In each case, the all-trans chain length corresponded to the total number of CH_2 groups. The 4-position shows significantly more disorder (about 11%) in the gel phase; a similar phenomenon was qualitatively noted by Bansil et al. (1980) in their Raman line-width study of the CD_2 stretching modes. Their explanation of inhomogeneous broadening related to the CO group is probably not applicable to the current data, which may reflect the bending of the *sn*2 chain in the interfacial region as seen in the crystal structure of DMPC (Pearson & Pascher, 1979).

Gaber et al. (1977) have suggested that from 15 to 32 °C an increase of about one gauche bond/chain is noted. This would translate to a change in the percentage gauche from 7.6 to 14.6% at the 4-position. We do note an increase from 7.6 to 10.9% over the temperature range 20–32 °C for 4-d_4-DPPC (Table I). However, the small fraction of gauche conformers in the gel phase of the 6- and 10-d_4-DPPC molecules suggests that any disordering in the gel phase is concentrated toward the bilayer center, as indeed has been suggested by Gaber and Peticolas (1977).

On the whole, the measurements reported here are in excellent agreement with previous NMR and dilatometric determinations of conformational disorder in bilayers. However, the current experiments offer distinct advantages compared with other techniques. First, the position dependence of the conformational disorder can be directly monitored, thus permitting evaluation of the effects of membrane-active species at particular depths in the bilayer. Second, the rocking modes that we have studied are sensitive only to trans–gauche isomerization in the acyl chains. Thus, we do not have to rely on elaborate theoretical models such as required in the analysis of NMR data (Westerman et al., 1982; Wittebort et al., 1987) to separate out the contributions of other motions to the chosen spectral bands. Finally, we can begin (Table II) to isolate the particular kinds of conformers that contribute to the disorder. The band at 646 cm^{-1} arises from the *tgg* class of conformers,

as defined under Theory, while the 652-cm^{-1} band arises from the *tgt* class. We note that kinks cannot be separated from single gauche rotamers.

The primary drawbacks to the current FT-IR experiment are the requirements for specifically deuterated material and the weakness of the IR absorption bands. The syntheses of these specifically deuterated PCs have been improved to the point where it is reasonable to generate 1–3 g of material (H. Schuster, S. S. Hall, and R. Mendelsohn, unpublished results). The weakness of the IR bands is the limiting factor in the ultimate utility of our approach. The bands (whose absorbance values at the path lengths used range between 0.040 and 0.001) sit on an intense background arising from solvent librational modes. Switching from H_2O to D_2O reduces the interference to a level of about 1.0–1.5 absorbance units at the path lengths utilized. Increasing the path length to increase the absorbance of the CD_2 groups raises the underlying solvent absorbance to an unacceptable level.

With current technology, it should be possible to examine the effect of small molecules such as steroids and peptides on the lipid conformational order. Finally, the quantitative determination of acyl-chain order reported should be a useful test for statistical mechanical theories of bilayer conformation [e.g., Pink et al. (1982)].

Registry No. 4-d_4-DPPC, 122844-70-6; 6-d_4-DPPC, 122844-71-7; 10-d_4-DPPC, 122844-72-8; DPPC, 2644-64-6.

References

Bansil, R., Day, J., Meadows, M., Rice, D., & Oldfield, E. (1980) *Biochemistry 19*, 1938–1943.

Cameron, D. G., Casal, H. L., Mantsch, H. H., Boulanger, Y., & Smith, I. C. P. (1981) *Biophys. J. 35*, 1–16.

Cameron, D. G., Kaupinnen, J. K., Moffatt, D. J., & Mantsch, H. H. (1982) *Appl. Spectrosc. 36(3)*, 245–249.

Chapman, D. (1975) in *Cell Membranes, Biochemistry, Cell Biology and Pathology* (Weissmann, G., & Claiborne, R., Eds.) HP Publishing, New York.

Fraser, R. D. B., & Suzuki, E. (1973) in *Physical Principles and Techniques of Protein Chemistry* (Leach, S. J., Ed.) Academic Press, New York.

Gaber, B. P., & Peticolas, W. L. (1977) *Biochim. Biophys. Acta 465*, 260–274.

Gruen, D. W. R. (1982) *Chem. Phys. Lipids 30*, 105–120.

Hinz, H. J., & Sturtevant, J. M. (1972) *J. Biol. Chem. 247*, 6071–6075.

Levin, I. W., & Bush, S. F. (1981) *Biochim. Biophys. Acta 640*, 760–766.

Lieb, W. R., Kovalycsik, M., & Mendelsohn, R. (1982) *Biochim. Biophys. Acta 688*, 388–398.

Maroncelli, M., Strauss, H. L., & Snyder, R. G. (1985a) *J. Chem. Phys. 82*, 2811–2824.

Maroncelli, M., Strauss, H. L., & Snyder, R. G. (1985b) *J. Phys. Chem. 89*, 4390–4395.

Mendelsohn, R., & Mantsch, H. H. (1986) in *Progress in Protein-Lipid Interaction 2.* (Watts, A., & de Pont, J. J. H. H. M., Eds.) Elsevier Science Publishers (Biomedical Division), Amsterdam.

Mushayakarara, E., Albon, N., & Levin, I. W. (1982) *Biochim. Biophys. Acta 686*, 153–159.

Nagle, J. F., & Wilkinson, D. A. (1978) *Biophys. J. 23*, 159–175.

Pearson, R. H., & Pascher, I. (1979) *Nature 281*, 499–501.

Petersen, N. O., & Chan, S. I. (1977) *Biochemistry 16*, 2657–2667.

Pink, D. A., Green, T. J., & Chapman, D. (1980) *Biochemistry 19*, 349–356.

Pink, D. A., Lookman, T., MacDonald, A. L., Zuckermann, M. J., & Jan, N. (1982) *Biochim. Biophys. Acta 687*, 42–56.

Seelig, A., & Seelig, J. (1974) *Biochemistry 13*, 4839–4845.

Seelig, J., & MacDonald, P. M. (1987) *Acc. Chem. Res. 20*, 221–228.

Shannon, V. L., Strauss, H. L., Snyder, R. G., Elliger, C. A., & Mattice, W. L. (1989) *J. Am. Chem. Soc. 111*, 1947–1958.

Snyder, R. G., & Poore, M. W. (1973) *Macromolecules 6*, 708–715.

Snyder, R. G., Cameron, D. G., Casal, H. L., Compton, D. A. C., & Mantsch, H. H. (1980) in *Proceedings VIIth International Conference on Raman Spectroscopy* (Murphy, W. F., Ed.) North Holland Publishing Co., Amsterdam.

Taraschi, T., & Mendelsohn, R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2362–2366.

Taylor, M. O., & Smith, I. C. P. (1980) *Biochim. Biophys. Acta 59*, 140–149.

Theimer, O. H. (1957) *J. Chem. Phys. 27*, 408–416.

Tulloch, A. P. (1979) *Chem. Phys. Lipids 24*, 391–406.

Vogel, H., & Jahnig, F. (1981) *Chem. Phys. Lipids 29*, 83–101.

Westerman, P. W., Vaz, M. J., Strenk, L. M., & Doane, J. W. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2890–2894.

Wittebort, R. J., Olejniczak, E. T., & Griffin, R. G. (1987) *J. Chem. Phys. 86*, 5411–5420.

Yellin, N., & Levin, I. W. (1977) *Biochemistry 16*, 642–647.