CD_2 Rocking Modes as Quantitative Infrared Probes of One-, Two-, and Three-Bond Conformational Disorder in Dipalmitoylphosphatidylcholine and Dipalmitoylphosphatidylcholine/Cholesterol Mixtures†

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ABSTRACT: The use of CD_2 rocking modes in the IR spectrum as quantitative probes of phospholipid conformational disorder has recently been described for aqueous dispersions of 1,2-dipalmitoylphosphatidylcholine (DPPC) and DPPC/cholesterol mixtures [Mendelsohn et al. (1989) *Biochemistry 28*, 8934–8939; Davies et al. (1990) *Biochemistry 29*, 4368–4373]. Initial studies focused at the 4, 6, and 10 acyl chain positions of DPPC. In the current work, the method is extended to the 2, 3, 12, and 13 positions. Conformational disorder in the L_α phase is approximately the same (about 20% gauche) at positions 4, 10, and 13, but an unexpected higher value is observed (about 30%) at the 6 position. Cholesterol (33 mol %) restricts gauche rotamer formation by factors ranging from 6 to 9 at positions 4 and 6, respectively, to 1.5–2 at positions 10, 12, and 13. Quantitative analysis for the DPPC/cholesterol "liquid-ordered" phase indicates the occurrence of 1.2 gauche bonds/chain, a marked reduction from the 3.6–4.2 gauche bonds/chain for DPPC alone. Proximity to the ester moiety at acyl chain position 3 perturbs the vibrational coupling patterns of the CD_2 rocking modes and eliminates their sensitivity to conformational change. In addition, the feasibility of a method based on the conformation-dependent coupling between CD_2 rocking frequencies of *two successive CD_2 groups* for the quantitative detection of specific, position-dependent king (gtg′) and isolated gauche (gtt) conformers is demonstrated. Finally, comparisons between IR measurements and explicit theoretical predictions of acyl chain conformational order are presented.

The quantitative determination of conformational disorder in the phospholipid acyl chains of biological membranes is essential both for elaboration of the fluid mosaic model and for the development of statistical mechanical theories of membrane structure. Toward this end, the quantitative application of Fourier transform infrared (FT-IR)[1] spectroscopy has recently been described (Mendelsohn et al., 1989). The method, based on the work of Snyder and Poore (1971), utilizes the CD_2 rocking modes of specifically deuterated phospholipids to probe trans–gauche isomerization at specific locations in lipid acyl chains. The approach depends on the fact that CD_2 rocking modes in a CH_2–CD_2–CH_2 skeleton appear at 622 cm^{-1} if the local geometry is trans but shift to about 650 cm^{-1} upon formation of gauche rotamers, with secondary shifts resulting from conformational effects farther along the chain. In previous studies, we have applied this approach to the 4, 6, and 10 positions in aqueous dispersions of DPPC (Mendelsohn et al., 1989) and DPPC/cholesterol mixtures (Davies et al., 1990a) and to the 6 position in DPPC/gramicidin mixtures (Davies et al., 1990b).

In the current work, the measurements have been extended to the bilayer center via syntheses of the 12-d_4 and 13-d_4 derivatives of DPPC and toward the polar headgroup via syntheses of the 2-d_4 and 3-d_4 derivatives of DPPC. The extent of conformational disordering, as observed from (i) the measured distribution of gauche rotamers and (ii) the two- and three-bond nonplanar conformers in DPPC and DPPC/cholesterol mixtures as obtained from CH_2 wagging modes (Casal & McElhaney, 1990; Senak et al., 1991), is compared with several theoretical descriptions. The feasibility of a method for distinguishing the contributions of kink (g′tg) from isolated gauche forms (gtt) to the disordering process at particular chain positions is also proposed and demonstrated.

Materials and Methods

Preparation of -d_4 Derivatives of DPPC. Derivatives of DPPC with single CD_2 groups at particular acyl chain positions were synthesized according to the general protocols of Tulloch (1979) with modification of some of the coupling steps (H. F. Schuster, S. S. Hall, and R. Mendelsohn, manuscript in preparation). Derivatives were fully characterized by NMR, IR, elemental analysis, and mass spectrometry.

Preparation of DPPC-7,8-d_8. DPPC-7,8-d_8 was prepared as follows. First, hexadecan-1-ol-7,7,8,8-d_5 was prepared by catalytic reduction of 7-hexadecyn-1-ol-1-*d* (Aldrich) by using the following procedure. The glassware was extensively dried before use. 7-Hexadecyn-1-ol (850 mg, 3.57 mmol) was dissolved in 5 mL of chloroform, and the hydroxyl proton was exchanged by treatment four times with 5 mL of D_2O (99.8 atom % deuterium, Aldrich). The organic layer was separated and dried over sodium sulfate. Removal of the solvents gave 7-hexadecyn-1-ol-*1-d*, which was dried. Tris(triphenylphosphine)rhodium chloride [60 mg, 0.066 mmol; prepared from triphenylphosphine and rhodium (III) chloride hydrate according to the procedure of Young et al. (1965)] was sus-

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[1] Abbreviations: FT-IR, Fourier transform infrared; DPPC, 1,2-dipalmitoylphosphatidylcholine; T_m, gel–liquid-crystalline phase-transition temperature; t, trans; g, gauche.

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pended in 15 mL of dry degassed benzene. The suspension was evacuated and flushed four times with deuterium gas, which was generated from the reaction of sodium (washed with dry ether and benzene) and methanol-d_4 (Cambridge Isotope Laboratories, Woburn, MA). A solution of 7-hexadecyn-1-ol-*1-d* (734 mg, 3.07 mmol) in 3 mL of dry degassed benzene was added. The reaction mixture was degassed and flushed three times with D_2 gas and then stirrred for 24 h at room temperature under an atmosphere of deuterium by using a balloon. After removal of the catalyst by filtration through Celite 545, the solution was evaporated on a rotary evaporator. The residue was purified by flash chromatography (elution with 7:1 hexane/ethyl acetate) to yield 654 mg (74%) of hexadecan-1-ol-*1,7,7,8,8,*-d_5 as a white solid; mp 47–48 °C; IR ($CHCl_3$) 3416 (OH), 2182, 2080, cm^{-1} (CD_2CD_2); ^1H NMR (200 MHz, $CDCl_3$) δ 3.62 (t, J = 6.55 Hz, 2H, CH_2OD), 1.49–1.59 (m, 2H, CH_2CH_2OD), 1.15–1.43 [m, 22H, $(CH_2)_{11}$], 0.88 (t, J = 6.40 Hz, 3H, ω-CH_3). Hexadecanoic-*7,7,8,8,*-d_4 acid was obtained by oxidation of the alcohol as follows. To 294 mg (1.2 mmol) of hexadecan-1-ol-*1,7,7,8,8*-d_5 in 50 mL of glacial acetic acid was added dropwise 0.6 g (6.0 mmol) of chromium trioxide in 8 mL of 90% acetic acid. The reaction mixture was stirred for 24 h at room temperature. Water was added and the product extracted with ether. The ether layer was washed with water and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 200:50:1 hexane/ethyl acetate/formic acid) to yield 272 mg (88%) of hexadecanoic-*7,7,8,8*-d_4 acid as a white solid; mp 54–55 °C: IR ($CHCl_3$) 2180, 2078 (CD_2CD_2), 1706 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$) δ 2.35 (t, J = 7.42 Hz, 2H, CH_2CO_2), 1.25–1.63 [m, 22H, $(CH_2)_{11}$], 0.88 (t, J = 6.12 Hz, 3H, ω-CH_3); MS of the methyl ester: m/z, 274.30, intensity 3.17; m/z, 273.30, intensity 1.10; m/z 243.30, intensity 1.65; m/z 242.30, intensity 0.54. The deuterated fatty acid was coupled to *sn*-glycerophosphocholine by using the following procedure. To a suspension of 106.0 mg (0.24 mmol) of *sn*-glycerophosphocholine, cadmium chloride complex (purchased from Sigma and then dried for 5 h over phosphorus pentaoxide under vacuum at 78 °C), 59.0 mg (0.48 mmol) of 4-(dimethylamino)pyridine, and 126 mg (0.48 mmol) of hexadecanoic-*7,7,8,8*-d_4 acid in 5 mL of freshly distilled alcohol-free chloroform was added 124 mg (0.6 mmol) of dicyclohexylcarbodiimide. The reaction mixture was stirred for 36 h at room temperature under nitrogen. Chloroform (10 mL) was added, and the mixture was filtered through a Celite 545 pad, which was washed with 10 mL of chloroform. Removal of the solvents gave a residue that was purified by flash chromatography (elution first with 100% chloroform, then with 90:10, 60:40, and 20:80 $CHCl_3/CH_3OH$ to yield a white solid). The suspended silica gel was removed by filtering a chloroform solution of the solid through a 0.45-μm Metricel filter three times. The product, 1,2-(dihexadecanoyl-*7,7,8,8,7′,7′,8′,8′*-d_8)-*sn*-glycerophosphocholine, was lyophilized with benzene, affording 128 mg (72%) as a white solid; $[\alpha]^{25}_D$ +5.31° (*c* 1.27, $CHCl_3/CH_3OH$ 1:1); IR ($CHCl_3$) 2179, 2077 (CD_2CD_2), 1735 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 5.24 (m, 1H, CH_2CHCH_2), 3.48–4.43 (m, 8H, $POCH_2CH_2N$, CH_2CHCH_2), 3.43 (s, 9H, $N(CH_3)_3$], 2.29–2.37 [m, 4H, $(CH_2CO_2)_2$], 1.61–1.71 [m, 4H, $(CH_2CH_2CO_2)_2$] 1.20–1.45 [m, 40H, $(CH_2)_{20}$], 0.92 (t, J = 6.56 Hz, 6 H, ω-CH_3). Analysis: calculated for $C_{40}H_{72}D_8O_8NP{\cdot}2H_2O$:C, 61.75; H, 10.88; P, 3.98. Found: C, 61.63; H, 10.85; P, 3.84.

FT-IR Spectroscopy. DPPC samples for FT-IR were prepared by mixing 15 mg of dry lipid and 15 μL of D_2O in a culture tube, which was then sealed. The sample was hydrated at 50 °C, with agitation, for at least 1 h. Samples containing cholesterol were prepared by mixing powdered lipid and sterol in a 2:1 lipid/sterol mole ratio. The solid mixture was dissolved in chloroform and the solvent evaporated under a stream of N_2 gas before addition of D_2O. The resulting suspension was contained between two AgCl windows by a 6-μm spacer. The edge of the window assembly was wrapped with Teflon tape to minimize dehydration during spectral acquisition. The entire assembly was then inserted into a variable temperature cell (Harrick, Inc., Ossining, NY).

Spectra were acquired on a Digilab FTS-40 spectrometer equipped with a TGS detector. Typically, 1024 scans were collected with two levels of zero filling and triangular apodization. The instrument resolution was 4 cm^{-1}.

Table I: Conformation Dependence of the CD_2 Rocking Modes in Isolated and Consecutive CD_2 Groups in DPPC[a]

isolated CD_2 group		consecutive CD_2 groups	
wavenumber	conformation	wavenumber	conformation
622	tt[b]	562	ttt
646	tgg	575	gtt
646	g′tgg	589	ttt ($CHDCD_2$) +gtg′
652	tgt	600	gtg
652	gtg′	640	ggt

[a] Adapted from the experimentally observed bands of Snyder and Poore (1971) and Maroncelli et al. (1985b). [b] The underlined bond pair surrounds the central carbon of $CH_2CD_2CH_2$ in the acyl chain.

Results

CD_2 Rocking Modes at Various Depths in DPPC and DPPC/Cholesterol Bilayers. Typical baseline-flattened spectra for the CD_2 rocking region of DPPC-*13*-d_4 and DPPC-*6*-d_4 above and below T_m are shown in Figure 1, panels A and B. The subtraction and data reduction procedures have been detailed earlier [Mendelsohn et al., 1989; Davies et al., 1990 (a,b)]. The main spectral feature at 622 cm^{-1} in Figure 1A is characteristic of tt conformations around a $-CH_2-CD_2-CH_2-$ skeleton at the 12–13 and 13–14 C–C bonds in each chain. Below T_m, most of the intensity is concentrated in this band and is indicative of highly ordered acyl chains. Above T_m, increased intensity is observed for a spectral feature at about 560 cm^{-1} that arises from two overlapped bands near 646 and 652 cm^{-1} (better resolved in Figure 1B), characteristic of particular gauche-containing conformers (Table I). Figure 1A also shows a CHD rocking mode (659 cm^{-1}) that arises from the presence of incompletely deuterated chains and contributes some intensity (which must be factored out) to the peak at 650 cm^{-1}. The relative areas of the 622-, 652-, and 646-cm^{-1} bands, obtained by curve-fitting the contour, are converted to a fraction of gauche rotamers at a particular chain position by the relationship derived by Maroncelli et al. (1985a,b):

$$\frac{N_g}{N_g + N_t} = \frac{1}{1 + (1/I_{exp})}$$

$$I_{exp} = \frac{[I(652) + I(646)]}{I(622)}$$

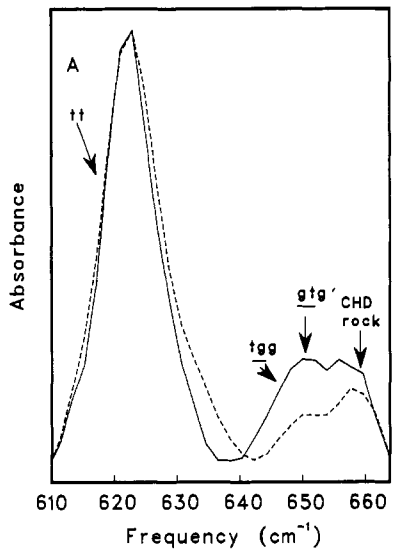
where N_g and N_t, are the number of trans and gauche bonds, respectively, and the I's are the areas under the indicated peaks.

The gauche rotamer percentages at specific chain positions obtained for the series of DPPC derivatives in the presence and absence of cholesterol (2:1 mol/mol, DPPC/cholesterol)

Table II: Position Dependence of Disorder in DPPC: Comparison of FT-IR with Theoretical Results

system method	DPPC FT-IR		DPPC/cholesterol FT-IR		DPPC theoretical[a]			
chain state conformation	g	g	g	g	g	$g_{90} + g_{120} + g_{180}$	t_0	t_{60}
temp (°C)	33	50	33	50	41	41	41	41
				Fraction of Indicated Conformer				
position								
4	0.11	0.21	0.02	0.03_7	0.29	0.02	0.38	0.31
6	0.02	0.30	0.03	0.03_6	0.28	0.02_5	0.42	0.28
10	0.03	0.20	0.03	0.13	0.28	0.03_8	0.39	0.30
12	nd[c]	nd	0.07	0.10	0.28	0.05_7	0.36	0.32
13	nd	0.17	0.05[d]	0.11	0.28	0.06_1	0.33	0.32
14/15[b]	nd	0.40	nd	nd	0.32	0.09_5	0.27	0.31

[a] Adapted from Meraldi and Schlitter (1981). See the text for definitions of configurations. [b] Determined (Casal & McElhaney, 1990) from the 1340-cm^{-1} wagging mode of end gauche conformers. [c] nd = not determined. [d] 20 °C.



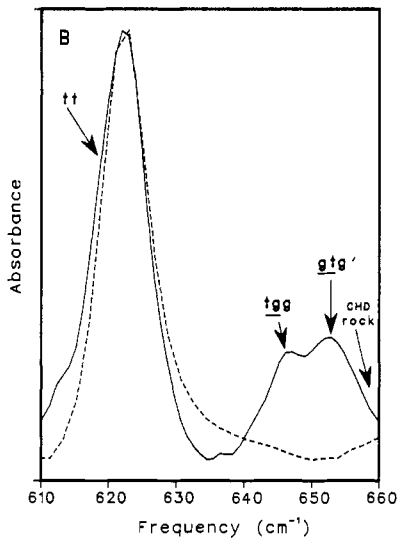


FIGURE 1: FT-IR spectra of the CD_2 rocking region (610–660 cm^{-1}) for DPPC-*13*-d_4 at 25 °C (---) and 50 °C (—) (A) and DPPC-*6*-d_4 at 25 °C (---) and 50 °C (—) (B). Particular conformer classes are indicated as follows: tt, trans; gtg', kink plus single gauche; tgg, multiple gauche. CHD rock refers to the rocking modes of incompletely deuterated groups. The contribution from the CHD rock is much greater for the *13*-d_4 derivative. The absorbance for the 622-cm^{-1} band at 50 °C for the 6 derivative is 18 mAU.

at temperatures above and below T_m are listed in Table II. The gel phase of DPPC appears highly ordered (trans conformation) at all positions except the 4 (4') position of the acyl chain, which demonstrates about 11% gauche bends. Similar

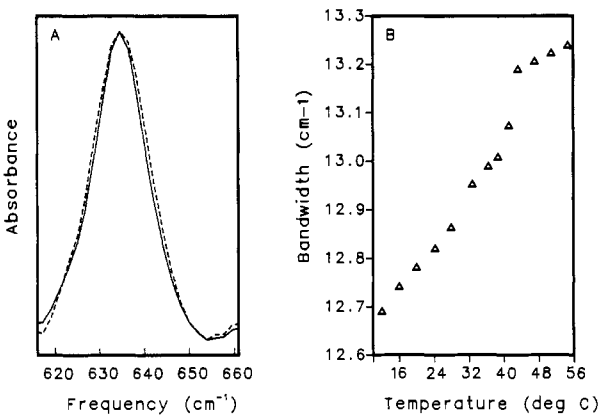


FIGURE 2: CD_2 rocking data for DPPC-*3*-d_4. (A) Spectra of the CD_2 rocking region of DPPC-*3*-d_4 at 25 °C (—) and 50 °C (---). Note the altered position of the rocking mode compared with Figure 1. (B) Temperature dependence of the line width of the CD_2 rocking mode for DPPC-*3*-d_4.

results (substantial gel phase disorder below T_m at the 4 and 4' positions) have been observed (Davies et al., unpublished results) for DPPE. At 50 °C, the 4, 10, and 13 positions of the liquid-crystalline phase show about 20% disorder, while toward the bilayer center at the 14–15 (end gauche) bond the number increases to about 40%. The latter value was obtained from the intensity of the CH_2 wagging modes near 1340 cm^{-1} of the end gauche bond of fully protonated chains (Casal & McElhaney, 1990). The observed 30% disorder at the 6 position is unexpected (a value closer to the 20% level was anticipated, by analogy with the data at the 4, 10, and 13 positions), yet the experiments have been repeated often over a 3-year period with different spectrometers and samples. We thus believe (unless some unexplained systematic error has affected our data reduction protocols) the difference to be a manifestation of somewhat fewer conformational constraints at this location.

Addition of cholesterol (2:1 DPPC/cholesterol, mol/mol) produces dramatic depth-dependent effects on disordering in the liquid-crystalline phase as summarized in Table II. At the 4 and 6 positions at 50 °C, the population of gauche rotamers is constrained to about 4% compared with 20 or 30%, respectively, in the absence of cholesterol. These constraints are much less severe at positions 10, 12, 13 where 10%–13% residual disorder is noted.

Spectra of the CD_2 rocking region for DPPC-*3*-d_4 above and below T_m are shown in Figure 2A. In contrast to the other derivatives (e.g., Figure 1), a single CD_2 rocking frequency at 635 cm^{-1} is noted at all temperatures. The line width of this feature responded to the thermotropic phase transition

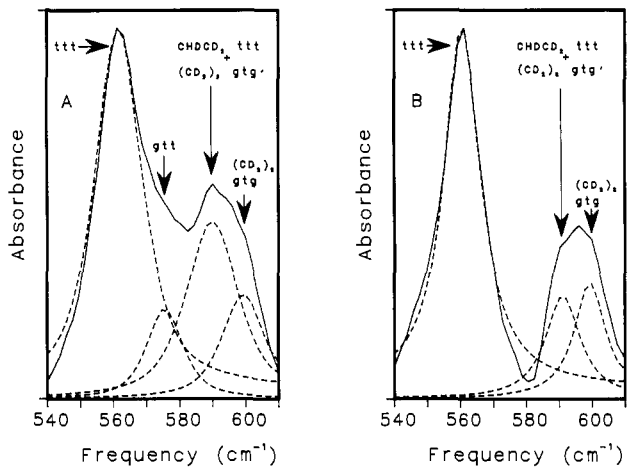


FIGURE 3: Spectra of the $-CD_2-CD_2-$ rocking region of DPPC-*7,8-d*$_8$. (A) L_α phase at 50 °C. The main subbands (dashed lines) were the result of an attempt to fit the contour to a minimum number of symmetric peaks. As the data are preliminary, no attempt was made to improve the fit with additional bands. (B) Gel phase at 25 °C. The main subbands (dashed lines) are also indicated.

(Figure 2B), but no intensity near 650 cm^{-1} was ever monitored. Finally, the CD_2 rocking bands of DPPC-*2-d*$_4$ could not be observed. Consistent with this observation is the reduced intensity of CD_2 stretching modes at this acyl chain position.

Coupled CD_2 Rocking Modes in DPPC-7,7,8,8,7',7',8',8'-d_8. The presence of consecutive CD_2 groups in an acyl chain results in a conformation-dependent coupling and splitting of the rocking modes (Table I) that permits the separate determination of kinks (gtg') and isolated gauche (gtt, for example) forms. The splitting however, also reduces the IR intensity of the individual components, rendering the experiment technically difficult. Nevertheless, reasonable, albeit preliminary spectra have been obtained (Figure 3) for DPPC-*7,7,8,8,7',7',8',8'-d*$_8$ (DPPC-*7,8-d*$_8$). Below T_m (Figure 3B) two main spectral features are noted. The feature at 562 cm^{-1} arises from ttt forms in the three bond $CH_2-CD_2-CD_2-CH_2$ central unit (Table I). The peak at 595 cm^{-1} arises from the overlap of two modes: a band arising from ttt forms in the $CH_2-CHD-CD_2-CH_2$ units of incompletely deuterated molecules and a band assigned to gtg forms. The significant contribution of the latter in the gel phase casts come doubt on the assignment of this feature. Above T_m, a new peak is observed at 574 cm^{-1} (Figure 3A), along with enhanced intensity at 589 cm^{-1}; these peaks correspond to the appearance of gtt and gtg' forms, respectively.

Discussion

The Disorder Profile. The importance of the current approach lies in the fact that trans–gauche isomerization, the most rapid motion available to the acyl chains, defines conformational disorder in lipid bilayers and hence determines the "instantaneous" state of organization of the membranes from which all other properties (longer time scales) follow. Of the myriad biophysical experiments applied to lipid systems, none defines this fundamental process as directly as the current FT-IR measurements. The quantitative aspects of these experiments are at a stage where they may be usefully compared with available theoretical descriptions.

A variety of theoretical and computational approaches have addressed the problem of gauche rotamer formation and its relationship to spectroscopically determined (usually NMR) order parameters (Schindler & Seelig, 1975; Meraldi & Schlitter, 1981; van der Ploeg & Berendsen, 1982; Gruen, 1982; De Loof et al., 1991). Schindler and Seelig (1975) applied the mean-field theory of Marcelja (1974) and calculated that for DPPC just above T_m 9.7 bonds are in the trans state and 4.3 bonds are in the gauche forms, in accord with measured range of 3.6–4.2 gauche bonds (Mendelsohn et al., 1989). More recent stochastic dynamic calculations predict 3.6 gauche bonds/chain (De Loof et al., 1991).

Table III: Overall Fractions of Particular Conformations in DPPC

	number of bonds in indicated state				
conformation	g[a]	gg[b]	g(90+120+180)[c]	kinks[c]	rel kinks + gtg[d]
temp (°C)					
50	3.9	0.36	0.67	0.52	1
60		0.42	0.75	0.52	0.9_5
70		0.58	0.83	0.50	0.9

[a] From Mendelsohn et al. (1989). Includes kinks, single gauche, and multiple gauche forms. [b] Senak et al. (1991). [c] Meraldi and Schlitter (1981). Determined from theoretical calculations. [d] Relative number of kinks + gtg calculated from the 1368-cm^{-1} intensity. The value at 50 °C is arbitrarily set to 1.0.

The current results for cholesterol/DPPC serve to delineate the molecular nature of the "liquid-ordered" phase proposed for this system (Ipsen et al., 1987). When averaged from position-dependent data in Table II, about 1.2 gauche bonds/chain are observed. This substantial reduction from DPPC alone delineates the strong conformational ordering induced by the sterol.

In discussions of order changes in lipid bilayers, it is necessary (Davis, 1983) to distinguish between molecular reorientations (as seen by ^2H NMR) and conformational changes (as seen by both FT-IR and ^2H NMR). The explicit position dependence of the probabilities of various configurations has been calculated by Meraldi and Schlitter (1981). Selected results are included in Table II. Their simulations suggest that loss of orientational order in the central part of the bilayer is due to a reduction in the number of (t, 0°) segments. In this notation, the angle referred to is the segment direction, i.e., that between the bilayer normal and the normal on the plane spanned by the two C–D bonds. Meraldi and Schlitter (1981) further note that increasing disorder toward the bilayer center arises from increases in the presence of both trans and gauche 90° and 120° conformers. These conformationally disordered states require large angular excursions from the bilayer normal and demand (at least) two gauche bends in the chain. Although these gauche bends may be widely separated in terms of of chain position (e.g., jogs), it is suggested that some of the configurations will involve consecutive gauche (gg or g'g') forms. The CH_2 wagging mode near 1353 cm^{-1} in fully proteated chains directly monitors the number of gg forms (Table III). McElhaney and Casal (1990) calculated 0.4 double gauche/chain at 45°, and Senak et al. (1991) showed that the number increases to about 0.6/chain at 70 °C. The parallel behavior as temperature is increased of the calculated (g, 90°) + (g, 120°) + (g, 180°) forms (Table III) and the experimentally determined double gauche forms is evident. The data, in conjunction with the calculations in Table II, suggest that the observed gg forms are concentrated toward the bilayer center.

Kink Formation and Determination by FT-IR. Early studies (Trauble, 1971; Marsh, 1974; Jackson, 1976) suggested that kink (gtg') formation is the preferred mode of acyl chain conformational disordering. In contrast, Meraldi and Schlitter (1981) and Ploeg and Berendsen (1982) calculated that kink sequences do not make a major contribution to the order parameter plateau observed in ^2H NMR experiments (Seelig

& Seelig, 1974) but that correlated (t, 60°) and (g, 60°) configurations dominate in this region. Meraldi and Schlitter (1981) further estimated the kink probability to be 0.03, approximately independent of position in the chain, with an overall expectation of about 0.5 kinks/C_{16} chain. This probability was calculated to be essentially independent of temperature from 41 to 80 °C. The contradictory nature of the theoretical predictions suggests that *experimental* resolution of this important problem is necessary. The number of kinks/chain and their distribution along the chain may be determined by FT-IR techniques, as noted below.

The overall kink concentration may be approximately obtained in fully protiated chains from the intensities of the localized CH_2 wagging mode near 1368 cm^{-1}. McElhaney and Casal (1990) have determined this number as 1.2 kinks/chain for DPPC at 5 °C above T_m, sufficient to account for the NMR order plateau. As discussed elsewhere (Senak et al., 1991), this estimate should be considered an upper limit, thus leaving the original problem unresolved.

Some insight to the position dependence of kink formation comes from the current studies of (single-position) specifically deuterated derivatives. The gauche band near 650 cm^{-1} is composed of a component at 652 cm^{-1} that arises from the sum of kinks and single gauche rotamers and a second feature at 646 cm^{-1} that arises from multiple gauche forms such as gtg'g' with the CD_2 group between the tg' bonds (see Table I). Mendelsohn et al. (1989) showed that the 652-cm^{-1} band is, not surprisingly, the prime contributor to conformational disordering in the L_α phase. However, since the band contains contributions from both isolated gauche and kink forms, these two factors cannot be distinguished, as is necessary for testing the theoretical models alluded to above.

The technically difficult solution to the problem of position-dependent kink concentrations has been pointed out by Snyder and Poore (1971). The approach requires synthesis of phospholipids with two adjacent CD_2 groups in the acyl chain. Under these conditions, the CD_2 rocking vibrations within each chain couple and produce modes sensitive to the local geometry around the $-CH_2-CD_2-CD_2-CH_2-$ skeleton (Table I). Although the feasibility of the experiment is clearly demonstrated in Figure 3, the data must be considered preliminary because the IR bands are weak. In addition, the original calculated values of Snyder and Poore (1971) for gauche-containing forms differ slightly from their (and our) observed frequencies. The assignments given under Results are thus considered tentative at this juncture. With these caveats, increases in both single gauche rotamer and kink sequences are evident above T_m with the increased intensity of bands at 574 cm^{-1} and 589 cm^{-1}, respectively. Quantitative analysis requires synthesis of appropriate alkanes and the application of the rotational isomeric state (RIS) model (Flory, 1969).

Finally, we have monitored the temperature dependence of the 1368 cm^{-1} kink + gtg marker band for DPPC (Table III). The relative intensity decreases slightly with increasing temperature. It is therefore concluded that the relative number of kink + gtg conformers also decrease with increasing temperature. These results are consistent with theoretical calculations (Meraldi & Schlitter, 1981) for DPPC.

Spectroscopic Behavior of DPPC-3-d_4. The CD_2 rocking modes exhibit their exquisite sensitivity to conformational changes because of the conformation-dependent coupling of this internal coordinate to adjacent CH_2 rocking modes. In the polar regions of the acyl chains, this favorable circumstance is altered. Spectra of DPPC-*3*-d_4 (Figure 2) in the rocking region demonstrate a main feature near 635 cm^{-1} that increases in half-width at T_m. There is no indication of a band arising from conformationally disordered chains near 650 cm^{-1} at the higher temperature. The band at 635 cm^{-1} is a CD_2 rocking mode that has shifted in frequency from either the tt (622 cm^{-1}) position or the gauche (646/652 cm^{-1}) doublet. The most likely possibility for both the change in frequency from the other derivatives and the absence of additional spectral features at high temperatures is a change in the coupling between the rocking modes of central CD_2 unit and the adjacent CH_2 moieties because of the presence of the ester group. Evidence that the normal coordinate has altered and that its conformation sensitivity has been eliminated comes from studies of DPPC in which the 3 and 3′ positions have been separately deuterated with CD_2 groups (H. Schuster and R. Mendelsohn, unpublished experiments). In each case a single feature at 635 cm^{-1} was observed both above and below T_m. Since the chain conformations at the 3 and 3′ positions are known to differ, the similarities in the CD_2 rocking bands in each case indicate that the conformational sensitivity of the rocking mode has been eliminated, i.e., the normal mode has changed. An additional possible origin of the frequency shift is the inductive effect which alters the CD_2 stretching frequencies (Bansil et al., 1980; Cameron et al., 1981). A progressive decrease in band frequencies as the CD_2 moiety is removed from the ester function levels off at the 6 position.

Conclusions

The CD_2 rocking modes in the IR spectra provide a unique solution to the problem of the determination of acyl chain conformational disorder and its position dependence in biological membranes. The overall occurrence of gauche forms is derived from the intensity of the 652/646-cm^{-1} doublet in chains with isolated CD_2 groups, while the specific occurrence of kinks vs single gauche bends is accessible from derivatives with consecutive CD_2 groups. The drawbacks of the approach are the need for extensive synthesis (technically feasible, but time-consuming) and the weakness of the spectral features, which leads to experimental uncertainties in the measured areas of weak bands. Enhanced detection sensitivity may alleviate the latter.

Registry No. DPPC, 63-89-8; 2-d_4-DPPC, 51799-37-2; 3-d_4-DPPC, 135193-34-9; 12-d_4-DPPC, 135193-32-7; 13-d_4-DPPC, 135193-33-8; cholesterol, 57-88-5; hexadecan-1-ol 1,7,7,8,8-d_5, 135193-29-2; 7-hexadecyn-1-ol-1-*d*, 135193-30-5; 7-hexadecyn-1-ol, 822-21-9; hexadecanoic-7,7,8,8-d_4 acid, 75736-49-1; *sn*-glycerophosphocholine, 28319-77-9; 1,2-(dihexadecanoyl-7,7,8,8,7′,7′,8′,8′-d_8)-*sn-glycero*-3-phosphocholine, 135193-31-6.

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Infrared Spectroscopic Studies on the Phosphatidylserine Bilayer Interacting with Calcium Ion: Effect of Cholesterol†

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ABSTRACT: Fourier transform infrared (IR) spectroscopic studies of phosphatidylserine/cholesterol/Ca^{2+} complexes are reported using the synthetic phosphatidylserines (PS) 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), and 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS). IR spectra reveal that cholesterol does not significantly alter the binding nature of Ca^{2+} to PS molecules; Ca^{2+} binds to the phosphate ester group of PS in the presence of cholesterol up to 50 mol % as in the case of pure PS bilayers. However, the IR data indicate that the presence of cholesterol induces disorder of the acyl chain packing, increases the degree of immobilization of the interfacial and polar regions, and increases the degree of dehydration of the PS/Ca^{2+} complexes.

The phospholipid bilayer is a major component of cellular membranes. It acts as a permeability barrier and a matrix where membrane proteins are embedded. Ca^{2+} is involved in the regulation of numerous cellular functions such as stimulus-contraction coupling, protoplasmic motility, intercellular interaction, and stimulus secretion coupling via the interaction with proteins which are embedded in the lipid bilayer (Siegel et al., 1980; Langer, 1987). The lateral distribution and functions of intrinsic membrane proteins are closely influenced by the physical and chemical nature of the lipid bilayer. The structure, phase behavior, and fusion of phospholipid membranes are affected by divalent cations, especially Ca^{2+}. Phosphatidylserine (PS)[1] is a major acidic phospholipid in mammalian plasma membrane (Rothman & Lenard, 1977; Devaux & Seigneuret, 1985). Due to its anionic character at physiological pH, it strongly binds with cations (Poste & Allison, 1973). It has been suggested that the interaction of this lipid with ions in the cytosol is central to a variety of physiological processes. In particular, the raised cytoplasmic Ca^{2+} levels predicted during exocytosis (Douglas, 1968) have been proposed to trigger the membrane fusion reaction by pathways involving ion association with PS (Gingell & Ginsberg, 1978).

The importance of the biological role of the Ca^{2+}/PS interaction has stimulated many structural studies on model membrane systems using various techniques (Seimiya & Ohki, 1973). The calcium ion induces crystallization of the acyl chains of PS, leading to an isothermal phase transition from the liquid-crystalline to the gel state (Papahadjopoulos et al., 1977). Infrared study (Dluhy et al., 1983) has revealed that Ca^{2+} binds to the phosphate ester (PO_2^-) group of PS and causes it to dehydrate. Ca^{2+} does not bind to the carboxylate (CO_2^-) group of PS nor does it dehydrate it. Instead, it immobilizes the CO_2^- group as well as the acyl chain group.

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[1] Abbreviations: FTIR, Fourier transform infrared; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine; *N*-methyl-DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-methyl-L-serine; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Chol, cholesterol; Tris, tris(hydroxymethyl)aminomethane hydrochloride; ESR, electron spin resonance; NMR, nuclear magnetic resonance.

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