Acyl chain conformational ordering in 1,2 dipalmitoylphosphatidylethanolamine

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

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ABSTRACT The extent of *trans*-gauche isomerization at the 4 and 4′ positions of the acyl chains of fully hydrated 4,4,4′,4′-d$_4$ 1,2-dipalmitoylphosphatidylethanolamine (4-d$_4$ DPPE) bilayers was quantitatively evaluated from the infrared (IR) intensity of the CD$_2$ rocking modes. About 20% gauche conformers were observed at 72°C (above T_m), while at 23°C, well below T_m, about 4% were noted. The order parameter $S_{C\text{-}D}$ was determined from ^{2}H nuclear magnetic resonance (NMR) quadrupolar splittings. $S_{C\text{-}D}$ is the product of a segmental order parameter (S_γ), which depends on conformational order, and a chain order parameter (S_α) which depends on slower motions such as chain wobble. The IR-determined percentage of gauche forms was converted into a segmental order parameter and factored out of the measured value for $S_{C\text{-}D}$ to yield an estimate of $S_\alpha = 0.59$ for L_α phase DPPE. A comparison with S_α for 1,2 dipalmitoylphosphatidylcholine (DPPC) suggests that increased wobble is responsible for enhanced motional averaging of the quadrupolar splittings in the latter at a similar reduced temperature. The extent of conformational disordering [at the 4(4′) position] is essentially unchanged between the two molecules. The current study demonstrates the advantage of integrating quantitative IR with ^{2}H NMR data, for elucidation of the contributions of the individual motions that average the NMR quadrupolar splittings.

INTRODUCTION

The quantitative determination of conformational states and dynamics in phospholipid acyl chains is a long sought goal of membrane biophysics. Toward these ends, Fourier transform infrared (FT-IR)[1] and ^{2}H NMR spectroscopies have proven to be important techniques. IR experiments can directly report on the extent of chain isomerization. Snyder and co-workers (1–3), in their seminal studies of alkane structure, demonstrated that the frequency of the rocking modes of isolated CD$_2$ groups in an otherwise fully proteated chain is sensitive to *trans*-gauche isomerization. In the first applications of this approach to biological systems (4), gauche conformer populations were determined in a series of specifically deuterated derivatives of 1,2 dipalmitoylphosphatidylcholine (DPPC). Acyl chain conformational disorder has also been determined in DPPC/cholesterol and DPPC/Gramicidin mixtures (5–7).

In contrast to the IR rocking modes whose positions respond mostly to *trans*-gauche isomerization in the acyl chains, ^{2}H NMR quadrupolar splittings are sensitive to long axis rotation and restricted wobbling motions of the molecular long axis as well as to *trans*-gauche isomerization in the chains, as long as the motional correlation times are in the intermediate or fast time regimes. All these motions also effect the ^{2}H spin-lattice relaxation times T_{1z} (8, 9).

To achieve a more complete description of acyl chain order and dynamics, we integrate data from FT-IR and ^{2}H NMR measurements on 4,4,4′,4′-d$_4$, 1,2 dipalmitoylphosphatidylethanolamine (4-d$_4$ DPPE). The incorporation of the quantitative measure of *trans*-gauche probabilities as obtained from IR into the total observed NMR order parameter $S_{C\text{-}D}$, allows a precise estimate of the acyl chain order parameter S_α.

MATERIALS AND METHODS

4-d$_4$DPPE and 4′,4′-d$_2$ DPPE were prepared as previously described (10). Fully proteated DPPE was obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Samples were prepared for FT-IR by the addition of 10 mg of phospholipid and 100 μl of D$_2$O to a culture tube which was then sealed. To ensure complete hydration, the sample was maintained at 70°C for 2–3 h with frequent agitation. The resultant creamlike suspension was contained with a 6-μM spacer between two AgCl windows. The windows were wrapped with Teflon™ tape to retard evaporation, and the entire assembly was inserted into a thermostated cell (Harrick Scientific, Ossining, NY).

IR spectra were recorded with either a Mattson Sirius 100 (Mattson Instruments, Inc., Madison, WI) FT-IR spectrometer equipped with a HgCdTe detector or a Digilab FTS-40 FT-IR spectrometer equipped with a TGS detector. 2000 (Mattson) or 1024 (Digilab) interferograms with a spectral resolution of 4 cm^{-1} were co-added, apodized with a triangular function, zero-filled (one level) and fast Fourier-transformed to produce a spectrum with data encoded every 2 cm^{-1}.

Details of the quantitative data analysis have been described elsewhere (4). Briefly, spectra of samples containing fully proteated DPPE and D$_2$O in the same proportions as in the specifically deuterated samples were recorded and processed as described above. These spectra in turn were used as backgrounds for spectral subtraction, necessary because the weak CD$_2$ rocking vibrations are superimposed on an intense D$_2$O libration background. After subtraction, the bands corresponding to the various conformational states were fit to a sum of Gaussian-Lorentzian sub-bands and the areas of the components were measured

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[1] *Abbreviations used in this paper:* 4-d$_4$ DPPE, 4,4,4′4′-d$_4$ 1,2-dipalmitoylphosphatidylethanolamine; DPPC, 1,2 dipalmitoylphosphatidylcholine; FT-IR, Fourier transform infrared; NMR, nuclear magnetic resonance; T_{1z}, spin-lattice relaxation time; 4-d$_2$ DPPE, 4,4-d$_2$ 1,2-dipalmitoylphosphatidylethanolamine.

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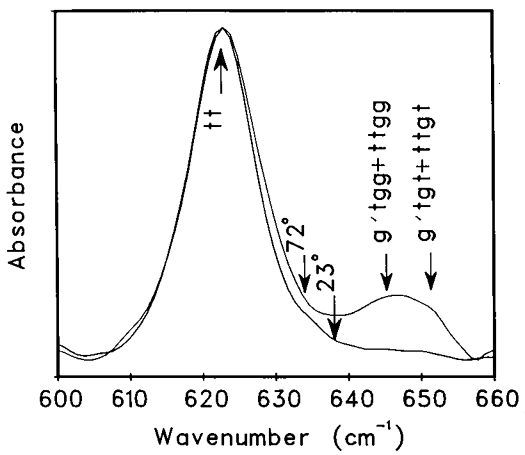

FIGURE 1 The CD_2 rocking region of the FT-IR spectrum for 4-d_4 DPPE at 72 and 23°C as indicated. The former has greater intensity in the 640–655 cm^{-1} region. The data have been normalized to the height of the 622 tt marker band. The conformational classes contributing to each band are defined in reference 3 and in the text.

using the elegant software generously supplied by D. Moffatt, National Research Council, Ottawa, Canada.

RESULTS

FT-IR spectra of the CD_2 rocking mode region in both the gel (23°C) and liquid crystal (70°C) phases for 4-d_4 DPPE (T_m = 64°C as determined from calorimetry) are shown in Fig. 1. Assignments of spectral features are taken from the work of Snyder and his associates (1–3). The band at 622 cm^{-1} arises from the rocking vibration of a CD_2 group adjoining two *trans* bonds. The broad contour centered at ~649 cm^{-1} is the superposition of two underlying sub-bands. The first, near 646 cm^{-1}, arises from CD_2 groups in multiple gauche conformations such as ttgg or g'tgg (The bond position in the sequence is underlined). The second, at ~652 cm^{-1}, arises from the sum of g'tgt (kink) or ttgt (single gauche) states. Extinction coefficients for the three marker bands are equal in alkanes (2, 3), so that the total fraction of gauche conformers is obtained by simply dividing the sum of the areas of the 646 and 652 cm^{-1} bands by the sum of these plus the *trans* (622 cm^{-1}) marker band area. Typical results of the curve-fitting algorithm used to determine band area ratios are shown in Fig. 2. The sum of the fit bands is virtually indistinguishable from the experimentally-determined spectrum. The fractional population of individual gauche rotamer classes in the L_α phase, taken as a percentage of the total gauche population, was obtained by dividing band areas of the individual markers by the sum of their areas. Table 1 lists the fraction of total gauche conformers in 4-d_4 DPPE at various temperatures as well the fractional population of individual conformer classes in the L_α state. The values of 0.8 for multiple gauche and 0.2 for kink plus single gauche were the fraction of the two disorder bands occupied by each individual band. Gauche marker bands below 45°C were very weak; the resulting reduced precision in the estimate of the gauche population is reflected in the stated uncertainty of the measurements in Table 1. The gauche fraction of 0.04 for the gel phase (e.g., 23°C in Fig. 1) comes from multiple determinations of the area of the two marker bands above a baseline drawn between spectral minima at 642 and 656 cm^{-1}.

DISCUSSION

Analysis of the IR data

As demonstrated previously (4–7), the conformational sensitivity of the CD_2 rocking modes makes possible a detailed mapping of the conformational states in phospholipid acyl chains. Because of the rapid time scale of molecular vibrations, the IR measurement is sensitive only to the *trans*-gauche isomerization process and detects distinct spectral features from each occurring conformer class. IR spectra yield the added dividend of providing direct information not only on the total fraction of gauche conformers present at a given chain position, but also partial data concerning the nature of the gauche state, e.g., kinds + single gauche states vs multiple gauche forms.

Analysis of the individual gauche marker bands (Table 1) for 4-d_4 DPPE reveals that a large fraction of the disorder in the L_α phase arises from multiple gauche forms. While the presence of such conformations may at first seem surprising [on the grounds that multiple gauche forms are disruptive to chain packing (11)], inspection of models constructed from x-ray data (12) reveals the importance of a ~90 degree bend at the C2′ position of the *sn*-2 chain in determining the molecular structure. This feature (in part) results from a static gauche bond that forms between C2′ and C3′, which we denote as g_s. It is also evident from model building that

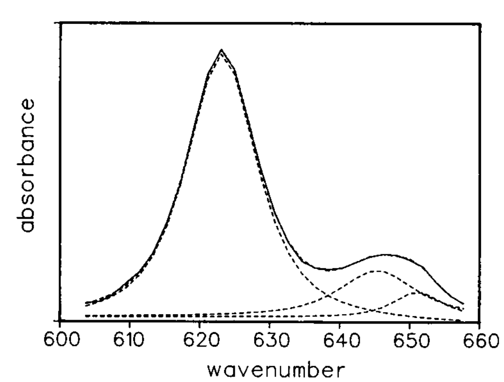

FIGURE 2 Results of the curve-fitting algorithm for determination of the contribution of the three constituent sub-bands to the contour between 600 and 660 cm^{-1}. The original spectrum is shown as a solid line. The three sub-bands are depicted as dashed lines. The sum of the sub-band contours is also depicted as a dashed line superimposed on (and virtually indistinguishable from) the original spectrum.

TABLE 1 **Gauche fraction and conformational classes in 4-d_4 DPPE**

	Fraction gauche		Conformational state	
			Multiple gauche	Kink + single gauche
T (°C)	IR[‡]	^{2}H NMR[§]	646 cm^{-1}	652 cm^{-1}
72	0.20 ± 0.01	—	0.8 ± 0.1	0.2 ± 0.1
67	0.17 ± 0.01	—	0.8 ± 0.1	0.2 ± 0.1
63	0.09 ± 0.03	—	—	—
60		0.07	—	—
45	0.04 ± 0.02	0.06	—	—
35		0.06	—	—
30		0.05	—	—
23	0.04 ± 0.02	—	—	—
23*	0.04 ± 0.02	—	—	—

* This datum is for 4′,4′-d_2 DPPE; [‡]this work; and [§]from Reference 20.

formation of kinks adjacent to the g_s structure (e.g., sequences of the type g_sgtg') is the mechanism of disorder that will introduce minimal excursion of the *sn*-2 chain from its original orientation in both gel and liquid crystalline phases. In terms of the IR spectroscopic measurement, kink formation (without a fixed gauche rotamer in the an adjacent C—C bond) normally produces a CD_2 rocking frequency of 652 cm^{-1}. In the presence of a fixed g_s bond, the vibrational frequency of the g_sgtg' form would appear as a multiple gauche state and shift to 646 cm^{-1}. Thus, a kink in the *sn*-2 chain encompassing C′ (3)-C′ (6) appears at 646 cm^{-1}, due to the spectral shift induced by the presence of the additional fixed gauche bond between C2′ and C3′. Kinks in the *sn*-1 chain are still expected to have their CD_2 rocking frequencies at 652 cm^{-1}. Attempts to explore this hypothesis quantitatively were frustrated by the presence of a weak spectral feature of unknown origin near 655 cm^{-1} in the L_α phase of fully proteated DPPE. This band rendered sufficiently precise quantitative background subtraction in spectra of 4,4-d_2 DPPE (above T_m) impossible. In 4-d_4DPPE, the disorder bands were sufficiently intense so that the precision in the subtraction operation was adequate.

Merging of IR with ^{2}H NMR results

To satisfactorily merge the IR and ^{2}H NMR data, the full set of physical processes that affect the latter spectral parameters must be considered. ^{2}H NMR measurements on gel phase 4-d_4 DMPE and 4-d_4 DPPE revealed that these molecules execute rotational jumps in the L_β-phase with rate constants for a threefold jump of $\sim 3 \times 10^6\ s^{-1}$. The reduced width of the ^{2}H-NMR powder patterns indicated that some residual *trans*-gauche isomerization is present, the gauche probability being ~0.05 at 30°C for DMPE as well as for DPPE (10, 13). The *trans*-gauche isomerization rate of $\sim 3 \times 10^5\ s^{-1}$ is in the intermediate exchange regime on the NMR time scale. As wobbling motions of the whole molecule are either absent or very slow, they do not contribute to the averaging process. The levels of residual conformational order as deduced separately from the IR and ^{2}H-NMR results for gel phase bilayers (Table 1) are in good agreement considering the assumptions and limitations of each experiment.

In the L_α phase, a direct comparison between the IR and ^{2}H NMR is more complicated as all averaging processes are fast on the NMR time scale. The quadrupolar splittings are narrowed by a factor of ~½ by fast long axis rotation, by *trans*-gauche isomerization and by orientational fluctuations of the long molecular axis (wobbling motion). L_α-phase ^{2}H NMR spectra of PC's and PE's revealed larger quadrupolar splitting for the PE's (10, 13–15). For instance, 4-d_4 DPPC at 51°C showed a quadrupolar splitting of 26.5 kHz compared to 29.5 kHz for 4-d_4 DPPE at 74°C. This indicates that the combined effects of conformational disorder and/or wobbling motions are reduced in DPPE compared with DPPC.

The potential for utilization of the IR data to separate the contributions of conformational disorder and wobbling to the NMR averaging process is possible because the CD_2 rocking modes are not sensitive to wobbling motions. The merging of the FT-IR results with the ^{2}H NMR data is accomplished as follows:

The total observed NMR order parameter $S_{C\text{-}D}$ is the product of a segmental order parameter S_γ and a chain order parameter S_α (16). From the IR-determined fraction of gauche conformers of 0.21 for DPPC (4) and the ^{2}H NMR-determined quadrupolar splitting of 26.5 kHz, a chain order parameter of 0.54 can be calculated for DPPC at 51°C, as $S_\gamma = -\frac{1}{2}p_t$ with $p_t = 1 - p_g$ (16). This result is in good agreement with chain order parameters determined solely by ^{2}H NMR using a combination of line shape simulations and relaxation time measurements (9, 17). The line shape model in the latter work is thus appropriate. Similar values of $S_\alpha = 0.58$ were reported for a fluorescent probe incorporated into DMPC bilayers above T_m (18). Finally, the current study for DPPE at 72°C (8° > T_m) yields $S_\alpha = 0.59$, significantly larger than for DPPC at 51°C, i.e., at a similar reduced temperature.

Two factors are evident from the combined IR/NMR

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data. First, conformational order in L_α phase of DPPE is at most slightly higher than in DPPC bilayers when compared on a reduced temperature scale, i.e., nine or ten degrees above T_m ($S_\gamma = -0.395$ and $p_g = 0.21$ for DPPC compared to $S_\gamma = -0.4$ and $p_g = 0.2$ for DPPE). In contrast, more significant differences are found for the chain order parameter S_α, which are 0.54 for DPPC compared to 0.59 for DPPE. It is expected that the difference in S_α values will become larger when the two systems are compared at the same absolute temperature, as the quadrupolar splittings are 29 kHz for DPPE compared to 21.2 kHz for DPPC at 75°C (15).

The integration of the IR data for conformational disordering with the ^{2}H NMR data produces a means of separating the relative contributions of *trans*-gauche isomerization in the acyl chains and wobbling motions of the entire molecule to the averaging process responsible for the reduced quadrupolar splittings. The reduced averaging observed for PE's compared with PC's arises mostly from a reduction of wobbling motions in the former. This is in accord with the observation that PE bilayers have a tendency to flatten out and not form small stable bilayer vesicles, a probable consequence of increased head group interactions via hydrogen bonds and a concomitant lower hydration of the ethanolamine head groups (19).

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