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Fluctuations in IR spectral parameters detected in mixed acyl chain membranes of *Acholeplasma laidlawii* B

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Abstract

Acholeplasma laidlawii B cells were grown at 37°C on three binary C16:0-d_{31}/C18:1 fatty acid mixtures at initial mol ratios of 3:2, 1:1, and 2:3. These mol ratios produced final C16:0-d_{31}/C18:1 lipid acyl chain mol ratios of 1.66 ± 0.23 ($n = 6$), 1.3 ± 0.20 ($n = 6$) and 0.58 ± 0.09 ($n = 10$), respectively, in the membrane of the microorganism. Membrane conformational order for the deuterated and proteated acyl chains in intact cells was monitored by FT-IR spectroscopy through the thermotropic response of the acyl chain CD_2 and CH_2 stretching frequencies. Intact cells and isolated membranes revealed broad phase transitions centered well below the growth temperature. This result differs from previous studies (Moore, D.J. and Mendelsohn, R. (1994) Biochemistry 33, 4080–4085) of cells grown on a single saturated fatty acid source, where T_m was close to the growth temperature. Fluctuations in IR spectral parameters from the liquid crystalline phases were detected in ten separate samples of cells grown on a 2:3 mixture (final mol ratio 0.58:1) of C16:0-d_{31}/C18:1, and in no other cell preparation. These were manifest by reduced precision in the measurement of CH_2 and CD_2 stretching frequencies and are attributed to fluctuations in the membrane conformational order. In addition to conformational order fluctuations in intact cells, similar behavior was noted for the simple binary phosphatidylcholine (PC) mixture, DOPC/1-C16:0-d_{31},2-C18:1 PC (2:1 molar ratio). In this instance, the fluctuations were also detected through the temporal and thermotropic response of the relative intensity of the 1341 cm^{-1} band assigned to end-*gauche* conformers about the penultimate C-C bond in the oleoyl chains. The relationship of these observations to the Raman spectroscopic detection of packing fluctuations in highly unsaturated PC's (Litman, B.J., Lewis, N., and Levin I.W. (1991) Biochemistry 30, 313–319) is considered.

Keywords: Membrane; Lipid conformation; FTIR; Lipid; Mycoplasma

1. Introduction

The mycoplasma *Acholeplasma laidlawii* offers several advantages compared with other cell types for physical studies of biomembrane conformational order and fluidity. Amongst these are the lack of a cell wall, the presence of a single (plasma) membrane and the ability of this organism to grow over a wide temperature range. Finally, this microorganism permits experimental control (within limits) of the membrane fatty acid composition [1].

A variety of physical and spectroscopic techniques have been utilized to study the *A. laidlawii* membrane including EPR, fluorescence, NMR, and FT-IR spectroscopies and DSC [2,3]. The techniques requiring exogenous, bulky probe molecules (fluorescence and EPR spectroscopies) have produced a variety of inconsistent results regarding the physical state of the cell membrane in these systems [4,5]. The non-probe techniques, FT-IR and DSC, have yielded consistent, complementary results regarding the thermodynamic behavior of isolated membranes and intact cells [6–9].

Abbreviations: DGDG, diglucosyldiacylglycerol; DOPC, 1,2-dioleoylphosphatidylcholine; DSC, differential scanning calorimetry; EPR, electron paramagnetic resonance; FT-IR, Fourier transform infrared; GC, gas chromatography; MGDG, monoglucosyldiacylglycerol; NMR, nuclear magnetic resonance; palmitic acid-d_{31}, acyl chain perdeuterated palmitic acid; PAPC, 1-palmitoyl-2-arachidonylphosphatidylcholine; PC, phosphatidylcholine; PDPC, 1-palmitoyl-2-docosahexaenoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PG, phosphatidylglycerol; $\nu_{asym}CD_2$, asymmetric CD_2 stretching frequency; $\nu_{asym}CH_2$, asymmetric CH_2 stretching frequency; $\nu_{sym}CD_2$, symmetric CD_2 stretching frequency; $\nu_{sym}CH_2$, symmetric CH_2 stretching frequency.

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Deuterium (^{2}H) NMR has been used in a few studies of isolated membranes and extracted lipids from cells grown on fatty acid mixtures containing C16:0-d_{31}. Two investigations have reported on membrane order in *A. laidlawii* cells grown on mixed saturated and unsaturated fatty acids [10,11]. These measurements were performed on isolated membrane pellets and extracted lipids. In both studies, cells were grown on fatty acid mixtures containing perdeuterated palmitic acid as one of the components. By using the C16:0-d_{31} acyl chains as reporter molecules, order parameters were derived for both isolated membranes and extracted lipids. The order parameters for membranes and extracted lipids were basically the same for any given system and decreased in membranes with a higher proportion of unsaturated acyl chains.

In recent years, FT-IR spectroscopy has developed as a significant biophysical technique for studying the conformational order of membrane lipid acyl chains in aqueous suspensions of live cells [8,9,12,13]. IR studies of mixed deuterated/proteated *A. laidlawii* cells complement and offer some advantages over the ^{2}H NMR studies. First, IR spectra are readily acquired from whole cell samples as well as isolated membranes. Second, as noted above, in the IR experiment the oleic and palmitic lipid chains are both directly monitored. Finally, the time scale of IR is significantly faster than acyl chain *trans/gauche* interconversion. This allows individual conformational states of the lipid acyl chains to be measured, thus providing a 'snapshot' of membrane conformation. In recent investigations of *A. laidlawii* B cells homogeneous in saturated fatty acids (C14:0, C15:0, or C16:0), this laboratory reported the detection of coupled CH_2 wagging modes, characteristic of all-*trans* acyl chains, in the IR spectra of whole cells and isolated membranes [8,9].

The current experiments extend the IR studies of *A. laidlawii* to cells grown on binary fatty acid mixtures of perdeuteriopalmitic (C16:0-d_{31}) and oleic (C18:1) acids. When supplied with such a fatty acid source in the presence of avidin, the cells incorporate both chains into its membrane lipids in a ratio close to that in the growth medium [10,14]. The frequency of the CH_2 stretching modes arising from the unsaturated lipid chains (18:1) can be measured in the same spectrum as the frequency of the CD_2 stretching modes from the saturated lipid chains (C16:0-d_{31}). As the frequencies of these modes are sensitive to acyl chain conformation [15], information is obtained directly about the conformational state of all acyl chains.

2. Materials and methods

2.1. Cell growth

A. laidlawii B cells were grown at 37°C in a lipid-depleted medium, pH 8.3, consisting of the following: Bacto Heart Infusion Broth (12 g/l), Bacto Peptone (5 g/l), Bacto Yeast Extract (5 g/l), glucose (2.5 g/l), Tris (3.5 g/l), bovine serum albumin (4 g/l), and penicillin G (10^5 units/l). Avidin was added to inhibit de novo fatty acid synthesis. Perdeuterated palmitic acid and oleic acid were mixed in initial mol ratios of 3:2, 1:1, and 2:3, and added to the growth media as concentrated ethanolic solutions. The final incubation concentration of fatty acid was 0.12 mM [14]. Cells were also grown on pure palmitic acid-d_{31}. At 37°C good cell growth was achieved both with the mixed fatty acids and pure perdeuterated palmitic acid. Virtually no growth was observed at 37°C with pure oleic acid, consistent with previous reports [10,14]. Lipid compositions were determined with GC analysis of some samples, and from the relative integrated intensities of the CD_2 and CH_2 and symmetric stretching modes (calibrated for different extinction coefficients with standard samples) for other samples. Consistent results were obtained from both methods.

2.2. Sample preparation and IR methods

Live cells were harvested in late log phase by centrifugation. Pellets of concentrated cells were placed between CaF_2 windows separated with a 12 μm spacer. FT-IR spectra were collected on a Mattson Instruments Research Series (RS-1) spectrometer equipped with an MCT detector. The CaF_2 windows enclosing the sample were contained in a thermostated transmission cell (Harrick Scientific, Ossining, NY). Temperature was controlled with a circulating water bath and monitored with a digital thermocouple (Physitemp Instruments Inc., Clifton, NJ). All spectra were obtained at 4 cm^{-1} resolution, under N_2 spectrometer purge, by co-addition of 1024 interferograms. The interferograms were apodized with a triangular function and Fourier-transformed with one level of zero filling to yield data encoded every ~ 2 cm^{-1}. All spectra were analyzed with software written at the National Research Council of Canada.

2.3. FT-IR data analysis

Spectra were collected from 5°C to 45°C at 3 degree intervals. In most cases samples were equilibrated for several minutes at each temperature before collecting spectra. In the experiments with cells grown on an initial 2:3 (palmitic/oleic) fatty acid ratio, several different protocols were followed; these are discussed in detail in Section 3. Briefly, in these experiments, samples were equilibrated for considerably longer time periods after which as many as 10 spectra were acquired at each temperature. As discussed below, the precision of these data were significantly different than the other data sets. In all cases the frequencies of the symmetric CH_2 and asymmetric CD_2 stretching modes were determined from second derivative spectra.

3. Results

As noted in Section 2, fatty acid compositions were determined either by GC or by IR determination of the integrated intensities of $\nu_{sym}CH_2$ and $\nu_{sym}CD_2$. Samples grown with initial mol ratios (palmitate-d_{31}/oleate) of 3:2, 1:1 and 2:3, revealed final mol ratios of 1.66 ± 0.23 ($n = 6$), 1.3 ± 0.2 ($n = 6$), and 0.58 ± 0.09 ($n = 10$), respectively. For the remainder of this report, these mol ratios are referred to as 1.7, 1.3 and 0.6, respectively. The excellent culture-to-culture reproducibility is indicated from the standard deviations. GC data also showed that C16:0-d_{31} and C18:1 acyl chains combined were more than 90% of the total acyl chain population. It is noted that full sets of temperature-dependent IR data were collected for all 22 samples for which analytical data are reported above. Completely consistent results were obtained.

Typical original and second derivative spectra of live *A. laidlawii B* cells with a final 1.3:1 (C16:0-d_{31}/C18:1) fatty acid composition are displayed in Fig. 1. The 3000–2800 cm^{-1} spectral region (Fig. 1A) shows $\nu_{sym}CH_2$ and $\nu_{asym}CH_2$ arising from the oleoyl acyl chains at ~2850 and ~2920 cm^{-1}, respectively. The 2250–2050 cm^{-1} region (Fig. 1B) shows $\nu_{sym}CD_2$ (~2090 cm^{-1}) and $\nu_{asym}CH_2$ (~2190 cm^{-1}) arising from palmitoyl-d_{31} acyl chains.

As a control for comparison with cells grown on fatty acid mixtures, the thermotropic response of $\nu_{asym}CD_2$ for cells grown on pure C16:0-d_{31} (proteated fatty acid level < 10% by GC and IR) is plotted in Fig. 2. These cells clearly undergo a gel-liquid crystal phase transition over the range 27–37°C centered at ~33–34°C (i.e., slightly below the growth temperature).

Fig. 3A shows the thermotropic behavior of $\nu_{asym}CD_2$ for cells grown with a final C16:0-d_{31}: C18:1 of 1.3:1. The phase transition detected is much broader than for the pure C16:0-d_{31} cells. In addition, the transition appears with

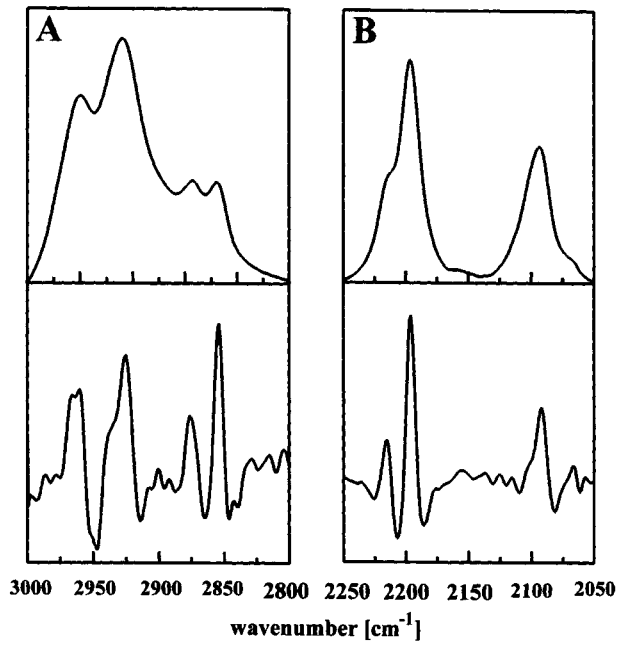

Fig. 1. (A) C-H stretching modes from the C18:1 chains of intact *A. laidlawii* cells enriched in C16:0-d_{31} and C18:1 fatty acids (1:1). The upper figure is a water-subtracted difference spectrum; the lower spectrum is a second derivative spectrum. (B) The C-D stretching modes from the C16:0-d_{31} membrane chains of the same cells as in (A) are displayed as both a water-subtracted spectrum (upper) and second derivative spectrum (lower). Second derivative spectra have been inverted.

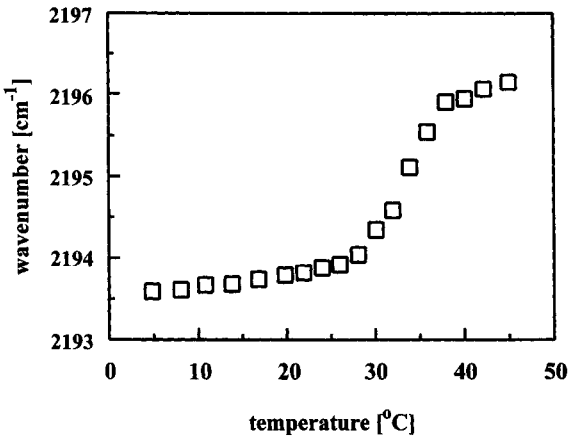

Fig. 2. The thermotropic behavior of the asymmetric CD_2 stretching frequency in pure C16:0-d_{31} enriched *A. laidlawii* cells. The T_m is ~33–34°C.

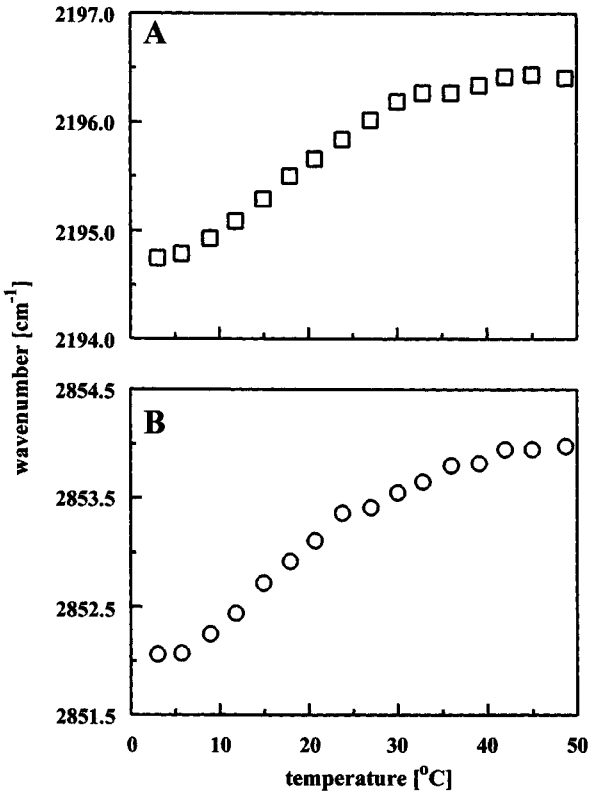

Fig. 3. The thermotropic response of the CD_2 asymmetric (A) and the CH_2 symmetric (B) stretching frequencies in intact *A. laidlawii* cells grown on a 1:1 ratio of C16:0-d_{31} and C18:1.

different completion parameters for palmitate-d_{31} and the oleate species. The palmitate-d_{31} acyl chain (Fig. 3A) has a well defined completion temperature at ~ 29°C, with an onset temperature not well defined because of the lack of points at temperatures < 0°C, while $\nu_{asym}CH_2$ for the oleate chain has a temperature profile (Fig. 3B) which demonstrates continuous curvature without a well-defined completion temperature. These data differ from some early DSC studies of McElhaney et al. [16] who reported a phase transition range of −11 to +8°C for cells possessing a palmitate/oleate ratio of ~ 50:50. In that study, the transition was partially obscured by the ice melting endotherm. In addition, cells were grown without avidin in the medium. These factors, coupled with the significantly higher palmitate levels in the current work, make direct comparisons between the two investigations difficult. The presence of excess palmitate in the current case would be expected to result in higher transition parameters, as indeed observed. The different IR behavior for the two spectral parameters may be the result of some (partial) phase separation for particular lipid species containing two palmitate chains.

$\nu_{asym}CD_2$ for cells grown on lipid mixtures is ~ 1 cm^{-1} higher at the lowest temperature (~ 5°C) compared with those of cells grown on pure palmitate-d_{31} (Fig. 2), indicating significantly more conformational disorder in the acyl chains of cells grown on lipid mixtures. At high

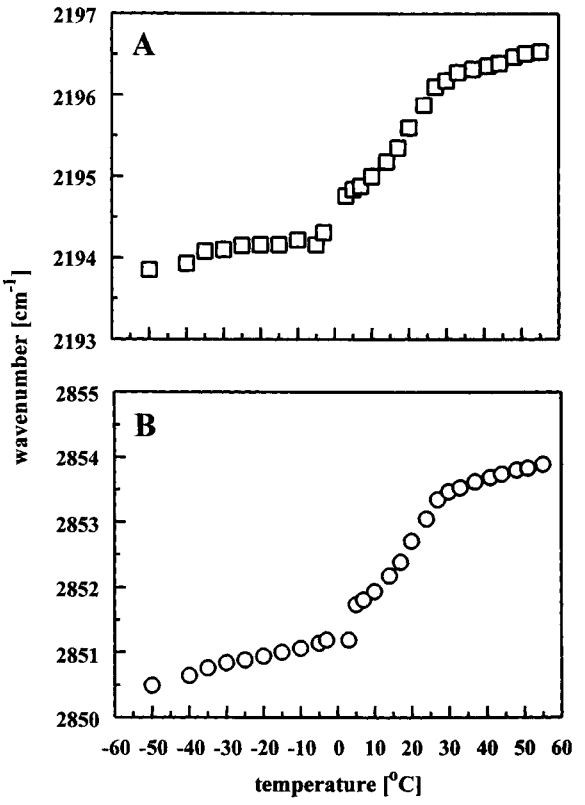

Fig. 4. The thermotropic response from −60 to +60°C of the CD_2 asymmetric (A) and the CH_2 symmetric (B) stretching frequencies in membranes isolated from *A. laidlawii* cells enriched in a 1:1 ratio of C16:0-d_{31} and C18:1.

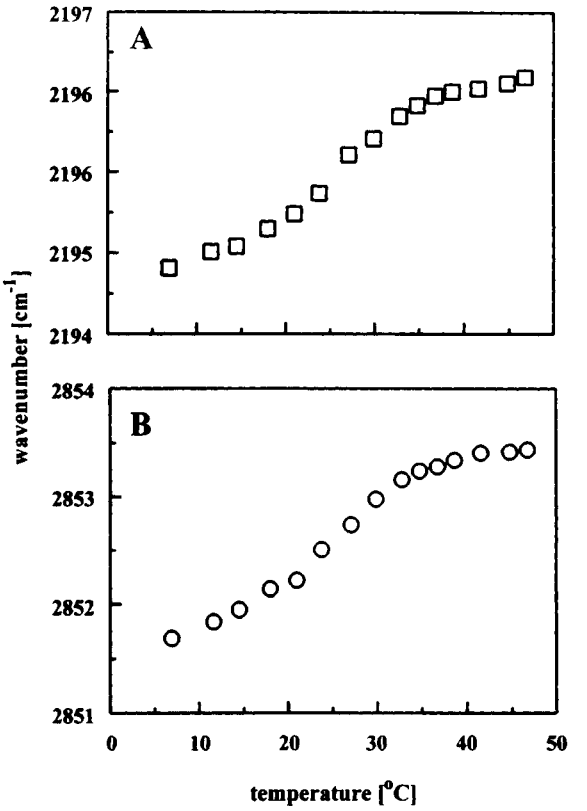

Fig. 5. The thermotropic response of the CD_2 asymmetric (A) and the CH_2 symmetric (B) stretching frequencies in intact *A. laidlawii* cells enriched in a 3:2 ratio of C16:0-d_{31} and C18:1.

temperatures (~ 45°C), the difference in frequency values between the pure C16:0-d_{31} and the 1.3:1 (C16:0-d_{31} and C18:1) cells is decreased to ~ 0.5 cm^{-1}, with the mixed system again being more disordered. The increased disorder probably results from poorer acyl chain packing induced by the presence of the oleate cis C = C bond.

To determine precise phase transition characteristics in the membranes of cells possessing a 1.3/1 ratio of C16:0-d_{31}/C18:1, spectra of isolated membranes were acquired from −60°C to +60°C. The thermotropic behavior of $\nu_{asym}CD_2$ and $\nu_{sym}CH_2$ from these experiments are plotted in Fig. 4A and B, respectively. The low temperature data make it clear that the *A. laidlawii* cell membranes with mixed acyl chains undergo a well-defined phase transition which begins ~ 0°C and ends at ~ 25°C, when measured by either of these IR frequency parameters. The end point of the transition is somewhat better defined for the oleate acyl chains in the membranes than in the live cells. The measured frequencies cover essentially the same range for each of the spectral parameters in the whole cells and membranes, suggesting no major differences in conformational order in going from the whole cells to the membranes.

The temperature dependencies of $\nu_{asym}CD_2$ and $\nu_{sym}CH_2$ from cells with a palmitate-d_{31}/oleate ratio of 1.7 are displayed in Fig. 5. Fig. 5A is a plot of the thermotropic

response of the asymmetric CD_2 frequency and Fig. 5B shows an equivalent plot for the CH_2 frequency. The transition parameters for the deuteriopalmitate chain are shifted to higher temperatures than for cells with a 1.3 palmitate-d_{31}/oleate ratio (compare Fig. 3A and Fig. 5A), consistent with a greater proportion of dipalmitoylated lipid species in the membrane. The completion temperature for the oleate species melting appears slightly lower than for the palmitate-d_{31} species.

The thermotropic responses of the methylene CH_2 and CD_2 stretching modes from *A. laidlawii* samples with a 0.6 fatty acid mixture (C16:0-d_{31}/C18:1) were significantly different from the 1.3:1 and 1.7:1 samples. The frequencies of the oleate CH_2 stretching vibrations as a function of temperature for the 1.3:1 and 0.6:1 (C16:0-d_{31}/C18:1) samples, are compared in Fig. 6A and B. The data for the 0.6:1 sample were recorded after an equilibration period of ~ 20 min at each temperature. All frequency values were measured for ten (10) consecutively acquired spectra at the given temperature. Thus cells were maintained at each temperature for ~ 1 h. This protocol was adopted after many experiments in which data were acquired in the usual manner, i.e., one spectrum per temperature. In such cases a substantial amount of 'scatter'

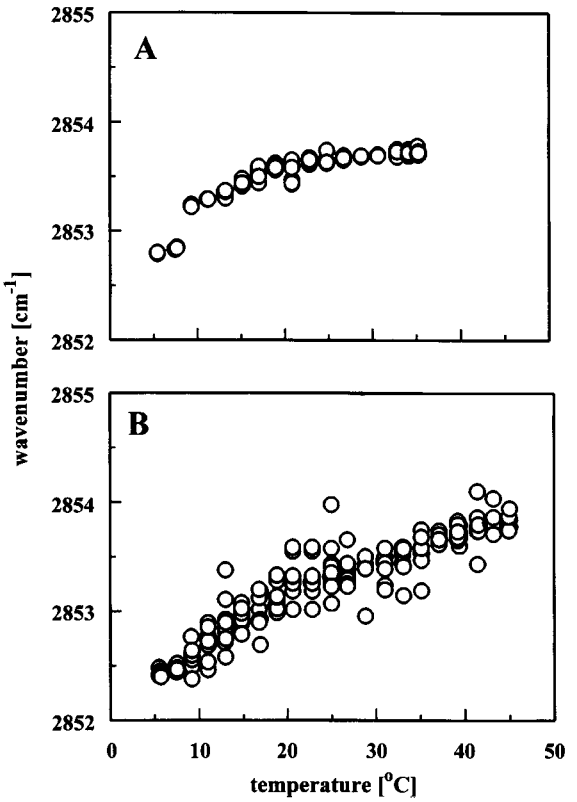

Fig. 6. Fluctuations in the CH_2 symmetric stretching frequency when multiple spectra (up to 10) are acquired at a given temperature (over the ~ 5–40°C temperature range) of intact *A. laidlawii* cells enriched in a 1:1 (A) or 2:3 (B) ratio of C16:0-d_{31} to C18:1. Significant 'scatter' (i.e., apparent loss of precision) is observed only when C18:1 chains are in excess (B).

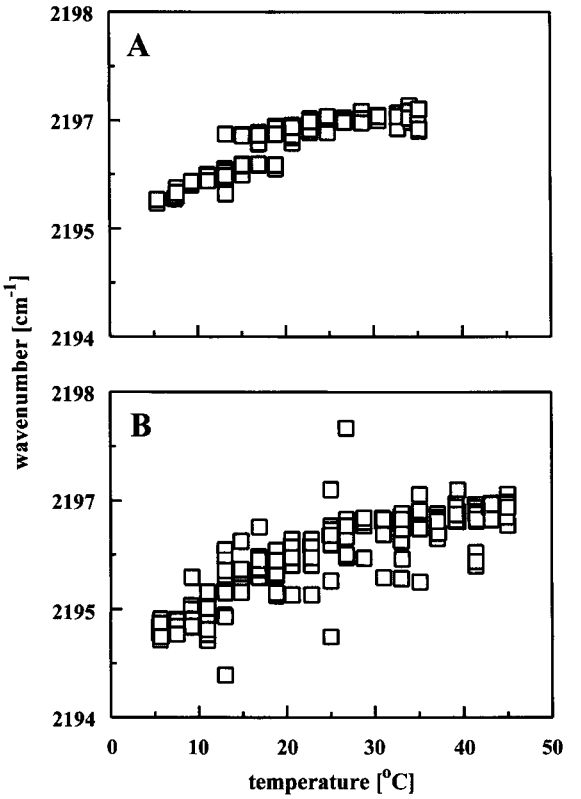

Fig. 7. Fluctuation in the CD_2 asymmetric stretching frequency when multiple spectra (up to 10) are acquired at a given temperature (over the ~ 5–40°C temperature range) of intact *A. laidlawii* cells enriched in a 1:1 (A) or 2:3 (B) ratio of C16:0-d_{31} to C18:1. As in Fig. 6, 'scatter' in the frequency values is observed only in the 2:3 (C16:0-d_{31}/C18:1) cells.

(i.e., an apparent loss of precision in the measurement) in the frequency values was routinely observed. Such 'scatter' was not observed in the other C16:0-d_{31}/C18:1 fatty acid mixtures nor in any of the previous experiments from this laboratory with *A. laidlawii* enriched in a single saturated fatty acid species [8,9]. Similar 'scatter' for the 0.6:1 compared with the 1.3:1 system is evident for $\nu_{asym}CD_2$ plotted in Fig. 7A and B. The variation in the frequency of the stretching vibrations, as noted for both the saturated and unsaturated acyl chains with this fatty acid mixture, was observed repeatedly in many replicate samples. Similarly, the normal (better) precision was always observed for the 1.7:1 and the 1.3:1 samples. These results are discussed below.

4. Discussion

A. laidlawii is known to incorporate unsaturated fatty acids (or lower T_m fatty acid) preferentially at the *sn*-2 position of the glycerol backbone [17,18] with the saturated species incorporating at the *sn*-1 position. In samples containing excess palmitate, a substantial proportion of dipalmitoylated species must occur. The melting of the

palmitate-d_{31} chains in the 1.3:1 and 1.7:1 samples reflects this effect as the palmitate transition parameters are raised by several degrees in the latter. When oleic acid is in excess (e.g., the 0.6:1 samples) a population of dioleoyl lipids must form. The head group (lipid class) composition has been reported to adapt to the needs of the organism and consists mainly of MGDG, DGDG, and PG [2,3,19,20].

A significant difference in the response of *A. laidlawii* to the mixed fatty acid source compared to the single fatty acid source was observed. In our previous studies [8,9], the growth temperature of the cells was close to the midpoint of the cooperative phase transition. In contrast, the phase transition in the mixed system is complete in all cases by the growth temperature and in many cases occurs substantially below the growth temperature. The cells grown on perdeuteriopalmitic acid exhibited (Fig. 2) a completion temperature within ~ 3 degrees of the growth temperature. This seems to represent an intermediate case.

The unanticipated result of the current experiments is the remarkable 'scatter' in both $\nu_{asym}CD_2$ and $\nu_{sym}CH_2$ in the 0.6:1 cells compared with the cells grown at the other two compositions. Interestingly, the average frequency values at the growth temperature for cells grown on 0.6:1 mixtures (Figs. 6 and 7) were not very different from those in the other mixed systems. This suggests that the overall average conformational order is unchanged between the various systems. The 'scatter' in frequency in the 0.6:1 system suggests that a dynamic process is occurring which produces significant oscillations in acyl chain conformation. When multiple spectra were acquired at a given temperature for the samples possessing excess palmitate-d_{31}, very little 'scatter' was observed. This was in agreement with all our previous IR studies of *A. laidlawii* and in agreement with hundreds of experiments on lipid model systems performed for a decade in this laboratory.

As this phenomenon is evidently unusual, several factors were considered to ensure that it is not an artifact of experimental technique or instrument malfunction. First, we note that samples displaying scatter and those displaying normal precision were examined in random temporal sequence over a period of one year. In all cases, samples of cells with C16:0-d_{31}/C18:1 ratios of 1.3:1 or 1.7:1 showed a precision typical of IR frequency measurements in live cells, i.e., scatter of ~ 0.1–0.2 for the majority component and scatter of ~ 0.3–0.4 cm^{-1} for the minority component. All ten samples with molar ratios of 0.6:1 showed scatter of ~ 0.7 cm^{-1} for the majority component and scatter of about 1 cm^{-1} for the minority. The range of variation for the latter at a given temperature occasionally reached 1.5 cm^{-1} (see Figs. 6 and 7). This range encompasses a wide variation in conformational disorder. Thus it is believed the 'scatter' in the 0.6:1 samples is not a simple consequence of instrument misalignment or drift during any particular thermotropic run. Second, spectral parameters at a given temperature were plotted as a function of time (data not shown). These showed random rather than cyclical variation, obviously for times longer than those needed for IR spectral acquisition (~ 5 min at any given temperature). Third, the variation is not a consequence of weak spectral signals, which could, in theory, reduce the precision of our frequency measurement. The scatter demonstrated in Figs. 6 and 7 is seen for both the majority (oleoyl) and minority (perdeuteriopalmitoyl) acyl chains. The magnitude of the absorbancies of these methylene stretches was about the same as in those samples which showed normal precision. Fourth, the experiments (cell growth, membrane isolation, spectral acquisition) were repeated six times for the 1.7:1 and 1.3:1 samples and ten times for the 0.6:1 sample. Completely consistent results were obtained. Finally, the frequencies were measured by several different algorithms available in this laboratory (second derivatives, center of gravity, with and without background subtraction, etc.). The precision levels in the measurement were unaltered by the choice of algorithm.

In view of the above controls, we were forced to the conclusion that we are examining a molecular phenomenon rather than an instrumental or a data reduction artifact. A time-dependent fluctuation in methylene frequency (reflecting a fluctuation in acyl chain conformational order) appears to be taking place. One previous report exists in the literature describing a similar phenomenon [21]. Litman et al. observed abnormal scatter in the temperature profiles constructed from Raman spectral parameters for 1-palmitoyl-2-arachidonylphosphatidylcholine (PAPC) and 1-palmitoyl-2-docosahexaenoylphosphatidylcholine (PDPC). In that instance, the Raman parameter depicting reduced precision was sensitive to packing arrangements of the chains [21].

To test for the existence of this phenomenon in systems simpler than whole cells, and in the hope of improving our overall experimental precision, we examined a 1:2 molar ratio mixture of 1,2-DOPC with 1-C16:0-d_{31}, 2-C18:1PC

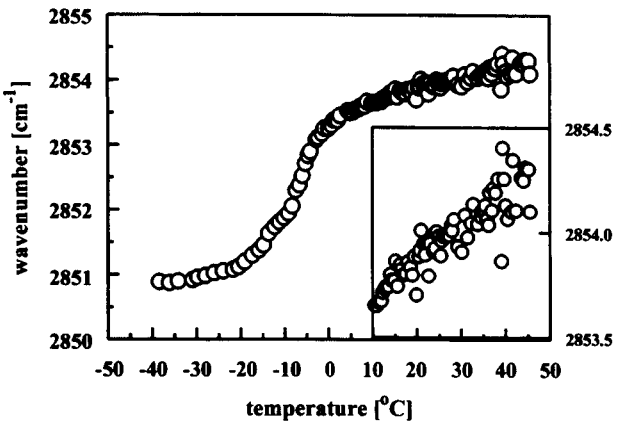

Fig. 8. Thermotropic behavior of the CH_2 symmetric stretching frequency in a 1:2 mixture of palmitate-d_{31}OPC and DOPC. Full precision (no 'scatter') is observed in the frequency values from −40°C to 15°C at which point substantial fluctuations commence. The inset amplifies the data over the region 10–50°C, to render the fluctuations more visible. Over 140 separate spectra were collected during this experiment.

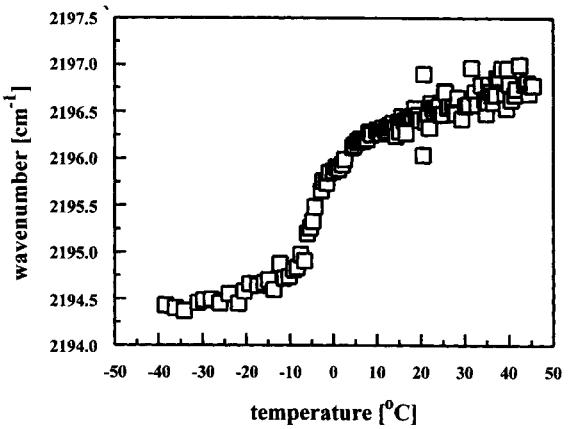

Fig. 9. Thermotropic behavior of the CD_2 asymmetric stretching frequency in a 1:2 mixture of palmitate-d_{31}OPC and DOPC. Full precision (no 'scatter') is observed in the frequency values from −40°C to 15°C at which point substantial fluctuations commence.

(P-d_{31}OPC). The thermotropic responses of the CH_2 and CD_2 stretching frequencies are shown in Figs. 8 and 9, respectively. A broad phase transition is evident in each case, with an onset temperature (difficult to measure precisely because of the curvature in the plots) of ~ −20°C for the oleate chains (Fig. 8) and somewhat higher (~ −8°C) for the palmitate-d_{31} chains (Fig. 9). Completion temperatures of between −5° and 0°C are seen in each instance.

Of major importance is the apparent variation in the precision of these measurements within a single experiment. For the deuterated component (Fig. 9), below 20°C, the precision of the measurement is better than about 0.1 cm^{-1}. Above 20°C, substantial scatter (0.3–0.4 cm^{-1}) appears in the data with an occasional range of 0.7 cm^{-1} at higher temperatures. Similar effects are noted for the CH_2 frequencies in the oleate chains. Thus the phenomena we are observing in *A. laidlawii* is not confined to intact cells, but may be monitored in phospholipid vesicles.

The variation in Raman parameters observed by Litman et al. in their studies of PAPC and PDPC were attributable to packing fluctuations [21]. The current IR parameters monitor conformational order fluctuations in essentially disordered phases. Further evidence for this was obtained from examination of the methylene wagging region (1330–1390 cm^{-1}) which provides a specific measure of the concentrations of some particular 2- and 3-bond conformational states in disordered phases of disaturated phospholipids [22]. Bands at 1341, 1353 and 1368 cm^{-1} in saturated acyl chains are assigned to end-*gauche* (e*g*), double *gauche* (*gg*) and the sum of kink + *gauche-trans-gauche* (*gtg*) states respectively [23]. Typical spectra for the current lipid mixture in the region 1330–1390 cm^{-1} at two temperatures $> T_m$ are shown in Fig. 10. The e*g* marker band is evident along with a feature at 1362 cm^{-1}, which has not been observed in spectra of disaturated species. That the origin of this vibration lies in the oleate chains is demonstrated in Fig. 11. In this figure, spectra of

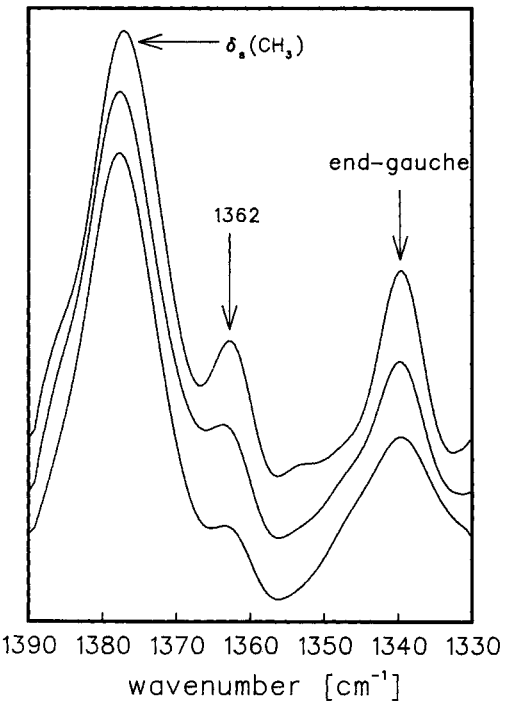

Fig. 10. The localized CH_2 wagging modes as detected in the original spectra of the 1:2 palmitate-d_{31}OPC/DOPC mixture. These spectra were acquired well above T_m (top), just above T_m (middle), and below the T_m (bottom). The assignment of these modes is discussed in the text.

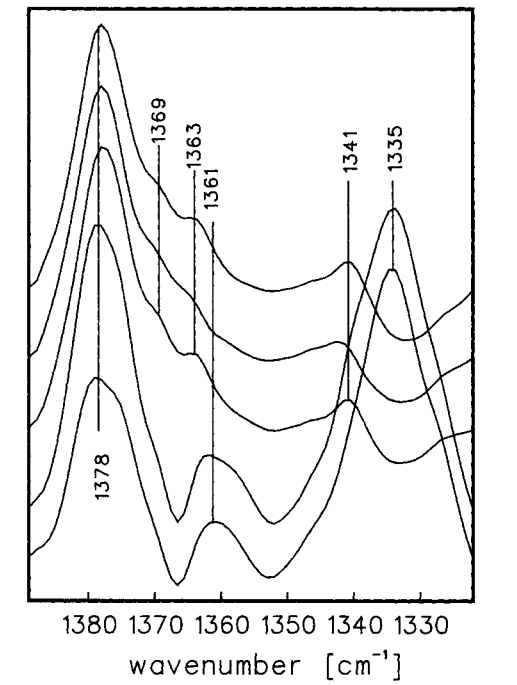

Fig. 11. The CH_2 wagging modes of DOPC plotted over a range of temperatures from −80°C to +30°C. T_m for this molecule is −22°C. Spectra below T_m have an intense feature at 1335 cm^{-1} and a medium intensity band at 1361 which vanish abruptly at T_m. Features at 1363 and 1369 cm^{-1} appear above T_m. The origin of these bands is discussed in the text. The temperatures for which data are plotted here are −80, −35, −7, 10 and +30°C.

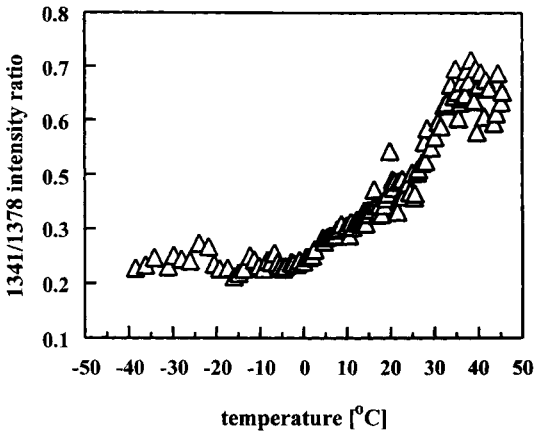

Fig. 12. The normalized intensity of the conformation-sensitive CH_2 wagging mode (arising from end-*gauche* conformers) as a function of temperature. Above ~ 20°C significant scatter is apparent in this measurement.

the wagging region of DOPC over a range of temperatures from −80 to +30°C are overlaid. The phase transition temperature of this molecule is ~ −22°C. A detailed discussion of the startling changes in the wagging spectral region is deferred to a later date. For the current purposes, we note that below T_m, the band at 1361 arises from the $k = 5$ component of the (coupled) methylene wagging mode progression. The assignment is unambiguous because of the simultaneous disappearance of the 1335 cm^{-1} band arising from the $k = 4$ progression component of the same progression. The wagging progression-based modes vanish near T_m and a new spectral feature at 1363 cm^{-1} becomes visible along with the kink + gtg marker band at 1369 cm^{-1}. This 1363 cm^{-1} feature is suggested to arise from methylene wagging modes of the C-C bonds adjacent to the C = C bonds, although the conformation giving rise to this feature is obscure.

In Fig. 12, the 1341 and 1362 cm^{-1} disorder bands for the binary lipid mixture are seen to increase in intensity with increasing temperature when measured relative to the temperature and conformation-invariant symmetric methyl deformation (umbrella) mode near 1378 cm^{-1}. The suggestion of fluctuations in conformational disorder toward higher temperatures is strengthened by examination of the variation in the normalized intensity of the *eg* marker band (Fig. 12). The scatter in the temperature-induced variation in this parameter is reduced below ~ 20°C. The behavior in precision parallels the thermotropic response of the methylene stretching frequencies (Figs. 8 and 9).

It is, of course, tempting to speculate on the origin of the observed fluctuations. First, the average values for both the CH_2 and CD_2 frequencies for which fluctuations occur (in live cells and in the lipid mixture) are consistent with the presence of substantial disorder in the chains. Second, the fluctuations require the presence of excess unsaturation. One possibility which has been discussed by Litman et al. is the existence of microscopic domains which fluctuate in size and produce oscillations in the observed Raman spectroscopic parameters [21]. These fluctuations may be driven by metabolic events in the whole cells or by instabilities in packing arrangements in the model system. Domains may tend to form because of preferential interactions between like chains (oleate-oleate or palmitate-palmitate). Segregation could produce regions of extremely poor acyl chain packing at the domain boundaries, a situation that might induce instabilities because of the ease of inducing conformational disorder to fill any voids. In any case, it is clear from the data in Figs. 10 and 11 that systems with high levels of chain unsaturation produce conformations not generally available to saturated acyl chains, as judged by the appearance of the 1362 cm^{-1} band in the former.

The main objective of the current study was to attempt to reliably demonstrate the occurrence of fluctuations. IR spectroscopy is by itself unable to pinpoint the structural origin of the effect. Other spectroscopic methods possessing inherently greater sensitivities, might be able to detect these fluctuations more rapidly than IR (current work) or Raman spectroscopies [21]. It takes a few minutes of data acquisition with either of these technologies to accumulate enough signal/noise in the measurements so that a reduction in the precision can be noticed at all. Techniques offering more rapid measurements might point to processes leading to these fluctuations.

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