Quantitative IR Studies of Acyl Chain Conformational Order in Fatty Acid Homogeneous Membranes of Live Cells of *Acholeplasma laidlawii* B†

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ABSTRACT: *Acholeplasma laidlawii* B has been grown highly enriched in myristic, pentadecanoic, and palmitic acids. The conformational order in the acyl chains of living cell membranes has been compared with that in the membranes of lysed cells and the lipids extracted therefrom, using Fourier-transform infrared (FT-IR) spectroscopic techniques. A gel–liquid-crystal-phase transition (25–45 °C) was noted for both the live cells and the membranes. Surprisingly, CH_2 wagging progessions, characteristic of coupled oscillators from acyl chains in the all-trans conformation, were detected in the live cells and in the cell membrane. A simple model suggested the presence of 1.7 gauche bonds/chain at the growth temperature (37 °C) in each case. Conformational order in the live cells and in the membranes was virtually identical over the range of cell viability (5–40 °C), as measured by the thermotropic responses of the $k = 1$ and $k = 2$ components of the wagging mode progression. This result was confirmed by studies of the thermotropic response of the symmetric CH_2 stretching vibrations, a qualitative index of acyl chain order. In contrast, the membrane lipid extracts (i) show much more conformational disorder from 5 to 25 °C than either the live cells or the membranes, (ii) undergo a gel–liquid transition over a broader temperature range and with a reduced magnitude of change in the symmetric CH_2 stretching frequency, and (iii) demonstrate a second transition centered at 50 °C, which is detected by a large increase in the *localized* CH_2 wagging mode (1368 cm^{-1}) that arises from conformationally disordered kink + gtg states. The current results are in good accord with some prior ^2H NMR and differential scanning calorimetry studies, but are in substantial disagreement with prior FT-IR investigations of similar systems. Possible reasons for the discrepancies are suggested.

The mycoplasma *Acholeplasma laidlawii* B has played an important role in studies of structure–function relationships in biological membranes [see reviews by McElhaney (1984, 1989) and Bittman (1993)]. This cell-wall-less procaryote may be grown over a range of temperatures and with a wide variety of fatty acids, many capable of incorporation at levels approaching near-homogeneity in the plasma membrane (Silvius & McElhaney, 1978; Silvius et al., 1980). The resulting membranes form useful systems for biophysical studies of molecular mechanisms of lipid organization, lipid/protein interaction, and responses of the plasma membrane to changes in the external environment.

Diverse biophysical techniques, including Fourier-transform infrared (FT-IR),[1] nuclear magnetic resonance (NMR), and electron spin resonance (ESR) spectroscopies, as well as differential scanning calorimetry (DSC) have been applied to the study of the *A. laidlawii* membrane. These, however, have produced inconsistent descriptions of thermotropic behavior and structural organization. A series of FT-IR studies (Cameron et al., 1982, 1985; Mantsch et al., 1988) showed substantial differences between the thermotropic properties of intact cells enriched in saturated perdeuteriated fatty acids compared with the isolated cell membranes, the former being substantially more disordered at all studied temperatures. ^2H NMR studies of live *A. laidlawii* B membranes have not been reported. However, isolated membranes enriched in [13-^2H]palmitic acid revealed a powder pattern at 45 °C characteristic of fluid lipid very similar to the lipid extract, without the presence of conformational order (Smith et al., 1979; Jarrell et al., 1982). The effect of proteins on the principal lipid-phase transition was thus minimal. Additional ^2H NMR studies (Stockton et al., 1975) suggested that at 37 °C, half the *A. laidlawii* membrane lipids are in an ordered state. The oft-contradictory ESR results have been summarized by McElhaney (1984).

Finally, conclusions that differ from the IR and NMR studies have been obtained from DSC studies of fatty acid homogeneous cells, membranes, and extracted lipids enriched in elaidic acid. The lipid organizations in intact cells and membranes were identical and quite different from those in the isolated lipids (Steim et al., 1969; Seguin et al., 1987).

Although some of the reported inconsistencies may be rationalized by the invocation of time-scale arguments, by probe-induced perturbations of the lipid organization, and by the fact that different techniques are sensitive to different aspects of lipid structure and dynamics, the situation remains confused.

For reinvestigation of *A. laidlawii* membranes, the experimental approach ought to provide characteristic signals that are directly interpretable in terms of well-defined membrane structural or dynamic parameters. Recently, two IR measurements that quantitatively probe conformational order in biological membranes have been reported. The first uses the intensity of the CH_2 wagging mode progressions (1190–1300 cm^{-1}) characteristic of, and very sensitive to, the presence of

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[1] Abbreviations: DSC, differential scanning calorimetry; ESR, electron spin resonance; FT-IR, Fourier-transform infrared; diC_{16}MGDG, 1,2-dipalmitoyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol; NMR, nuclear magnetic resonance; T_m, gel–liquid-crystal-phase transition temperature.

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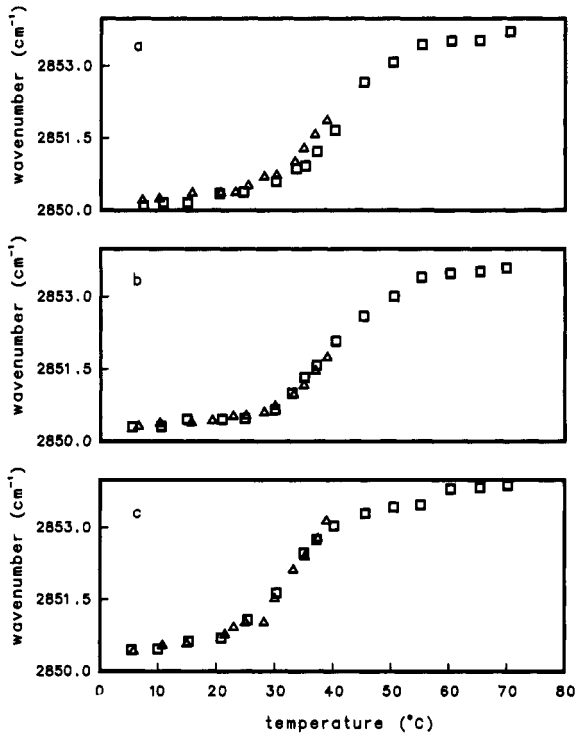

FIGURE 1: Thermotropic behavior of *A. laidlawii* as monitored through the CH_2 symmetric stretching frequencies. (a) C_{14}-enriched cells: (△) spectra collection stopped at 39 °C (to test for viability); (□) spectra collected up to 70 °C. (b) and (c) are the equivalent data for C_{15}- and C_{16}-enriched cells, respectively.

all-trans acyl chains (Snyder, 1960; Snyder & Schachtschneider, 1963; Senak et al., 1992; Chia & Mendelsohn, 1992). The second monitors the isolated CH_2 wagging modes (1330–1370 cm^{-1}) that characterize disordered phases (L_α, H_{II}, etc.) to detect the occurrence of specific two- and three-bond conformational states in the chains (Casal & McElhaney, 1990; Senak et al., 1991). In the current study, these approaches are applied to quantitatively compare acyl chain conformational order in *A. laidlawii* B cells enriched to near-homogeneity in C_{14}, C_{15}, and C_{16} chains, in their isolated membranes and in lipids extracted therefrom. Some of the earlier contradictions have been reconciled.

MATERIALS AND METHODS

Cell Growth. Acholeplasma 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 (Silvius & McElhaney, 1978). Pentadecanoic, myristic, or palmitic acids were added as concentrated ethanolic solutions to give a final concentration of 120 μM. Cells were harvested by centrifugation; concentrated cell suspensions were used for IR experiments. The pellet of live cells was placed between two CaF_2 windows separated with a 12-μm Teflon spacer. On several occasions, the viability of the live cells was confirmed by ending an IR experiment at 39 °C, returning the cells to agar plates, and reculturing the cells at 37 °C. Most IR experiments with live cell pellets were taken to 70 °C, at which temperature the cells are no longer viable. Figure 1a overlays the thermotropic response of the symmetric CH_2 stretching frequency of a myristic acid enriched live cell pellet taken to 39 °C with that of a second pellet, from the same cell culture, taken to 70 °C. Figure 1b and Figure 1c are the equivalent data for pentadecanoic acid and palmitic acid enriched live cells, respectively. The reproducibility of these results within each of the three systems along with the viability studies provide compelling evidence that at temperatures below about 40 °C (even in those experiments taken to 70 °C) our spectra are derived from live cells.

Membrane Preparation. To prepare membranes, the cells were lysed by osmotic shock. After three washes with distilled water, the membranes were concentrated by centrifugation, lyophilized, and stored in a freezer. Hydrated membrane samples for IR spectroscopy were prepared as follows: 200 μL of water was added to 5 mg of lyophilized membrane, which was then centrifuged and the supernatant removed. The membrane pellet was placed between two CaF_2 windows separated with a 6-μm Teflon spacer.

Membrane lipids were extracted according to Bligh and Dyer (1959). For IR experiments, the lipid extracts were hydrated in sealed ampules and taken through repeated heating and cooling cycles. Samples were placed between two CaF_2 windows separated by a 6-μm Teflon spacer.

For analysis of membrane acyl chain composition, the methyl esters were prepared by refluxing the lipid extract with methanolic HCl. These were then extracted with hexane. A Hewlett Packard 5890A gas chromatograph with a 30 m × 0.2 mm i.d. SP2380 column and helium carrier gas was used to analyze the methyl esters. The degree of fatty acid incorporation was always greatest for the pentadecanoic acid enriched cells, where the homogeneity was routinely above 90%. This level of homogeneity was also achieved with C14:0- and C16:0-enriched cells although not consistently. At these levels of homogeneity of saturated acyl chains, oxidation of membrane components during extraction protocols is not a concern. Oxidation products could not be detected in gas chromatography experiments. The diC_{16}MGDG [1,2-di-palmitoyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol] used in this study was the generous gift of Dr. Alfred Blume.

IR Methods. FT-IR spectra were collected with either a Digilab FTS-40 spectrometer or a Mattson Instruments Research Series (RS-1) spectrometer. Equivalent results were obtained with each instrument. 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) placed adjacent to the focus of the IR radiation. For experiments at temperatures below 0 °C, spectra were acquired in a liquid N_2 cooled dewar equipped with external AgCl windows. The sample was enclosed between CaF_2 windows and placed in a brass cell. The external AgCl windows and the brass cell could be separately heated. Temperature was regulated by the controlled boil-off of liquid N_2 and monitored with a thermocouple. Spectra were obtained at 4-cm^{-1} resolution, under N_2 purge, by co-addition of 256 (Digilab) or 1024 (Mattson) 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 transferred to an IBM-compatible microcomputer and analyzed using software supplied by D. Moffatt of the National Research Council of Canada.

FT-IR Data Analysis. Quantitative analysis of the CH_2 coupled wagging modes was accomplished as follows: The spectrum obtained at 70 °C was subtracted from spectra acquired at lower temperatures by choosing a subtraction factor that maximized the progression band heights while

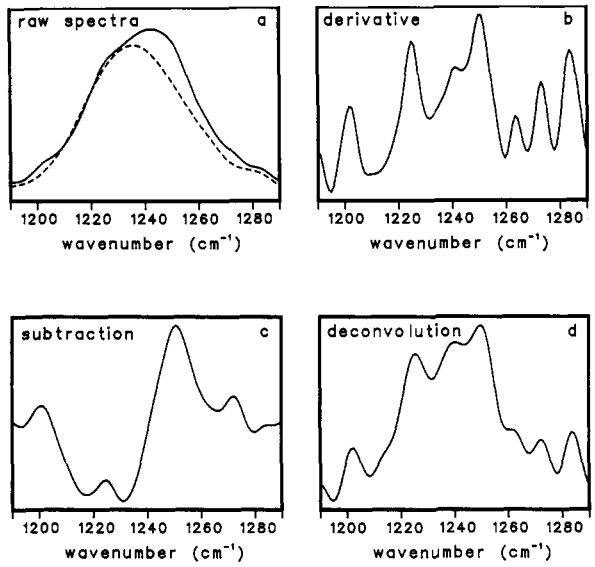

FIGURE 2: CH_2 wagging region (k = 1–4) of C_{15}-enriched live cells with underlying phosphate and protein bands subjected to various data reduction protocols as follows: (a) Raw spectra of C_{15}-enriched live cells at 5 °C (—) and 70 °C (- - -). (b) Second-derivative spectrum of C_{15}-enriched live cells at 5 °C. (c) Difference spectrum resulting from subtraction of the 70 °C spectrum from the 5 °C spectrum. (d) Deconvolution of the 5 °C live cell spectrum. Parameters for various data reduction protocols are discussed under Materials and Methods.

giving a consistent base line contour for a given spectral series. The difference spectra were then integrated between minima to give the intensity of the individual progression bands. Whereas in our previous studies of progression bands (Senak et al., 1992) the intensities were normalized to the underlying phosphate band, that procedure was unsuccessful for this series of experiments. The band underlying the 1230–1260-cm^{-1} region arises from several sources and varied somewhat as a function of temperature. Given this caveat, it is noted that the intensity of the k = 3 progression band, in both the live cells and the hydrated membrane, behaves similarly to the other progression bands. However, since there is no strong underlying band in the 1190–1230-cm^{-1} region, the k = 1 and k = 2 progression bands were routinely selected as indicators of acyl chain conformation. To enhance the level of confidence in assignment of the progression bands from difference spectra, we have further analyzed the raw spectral data with both second-derivative and Fourier self-deconvolution (Kauppinen et al., 1981) techniques. For second-derivative spectra, a break point of 0.6 was used, while for deconvolution, a half-width of 18 cm^{-1} and a resolution-enhancement factor (ratio of bandwidths before and after deconvolution) of 2.2 were optimal. Figure 2 illustrates clearly that the progression bands can be observed with second-derivative, deconvolution, or difference spectroscopy. Although the relative intensities of the progression bands are unlikely to be preserved in second-derivative data, the frequencies of the CH_2 wagging modes are easily measured and compared to reference compounds in Table I. To determine the frequency of the CH_2 symmetric stretch, water was subtracted from each spectrum over the 2800–3000-cm^{-1} region. The frequencies of the CH_2 stretching modes were then calculated using a center of gravity algorithm to give band positions with an uncertainty of less than 0.1 cm^{-1} (Cameron & Moffatt, 1984).

RESULTS

IR spectra of live *A. laidlawii* B cells and hydrated lyophilized membranes are inherently very complex, yet much

Table I: Wagging Mode Progression Frequencies (cm^{-1}) in Live Cells, Extracted Membranes, and Model Compounds

progression component	k = 1	k = 2	k = 3	k = 4
$diC_{14}PC$	1203.7[a]	1228.7	1255.7	1280.8
C_{14} live cells	1204.5	1227.7	1255.3	1281.1
C_{14} membrane	1203.5	1228.6	1255.3	1280.7
$diC_{15}PC$	1201.7	1224.9	1250.0	1273.1
C_{15} live cells	1201.8	1224.4	1248.8	1272.8
C_{15} membrane	1202.2	1225.0	1250.3	1273.0
$diC_{16}PC$	1199.8	1221.0	1244.2	1265.4
C_{16} live cells	1198.7	1222.0	1245.1	1264.8
C_{16} membrane	1198.6	1222.8	1245.0	1265.8
$diC_{16}MGDG$	1198.5	1221.1	1245.0	1266.2

[a] Wavenumber units (cm^{-1}).

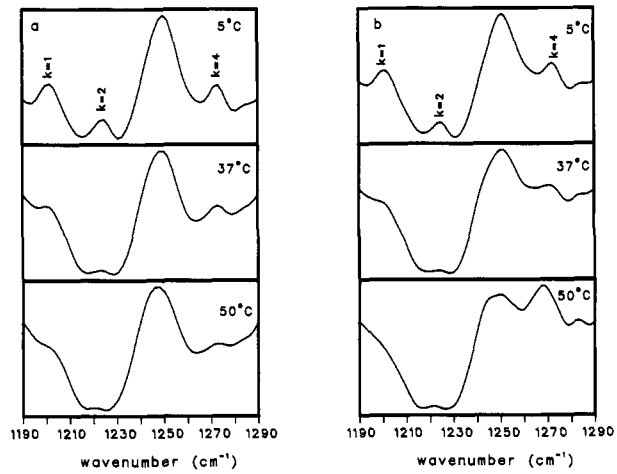

FIGURE 3: (a) Difference spectra of C_{15}-enriched live cells at 5, 37, and 50 °C over the progression band region 1190–1290 cm^{-1}. Note that the progression bands are still present, although weak, at the growth temperature. (b) Difference spectra of the C_{15}-enriched membrane at 5, 37, and 50 °C over the 1190–1290-cm^{-1} region.

useful information can be extracted through careful and consistent data reduction protocols. Figure 3 shows difference spectra for the CH_2 wagging region for C_{15}-enriched live cells and extracted membranes, each at 5, 37, and 50 °C. The k = 1, k = 2, and k = 4 components of the CH_2 wagging progression (see Table I) are clearly evident in the spectra. These diminish in intensity with increasing temperature. The k = 3 component (1255 cm^{-1}) is highly overlapped by an underlying band arising from the phosphate PO_2^- asymmetric stretch or protein amide III and is unsuitable for quantitation. The k = 4 mode in the membrane extract is also inappropriate for quantitation due to the appearance at 50 °C of a new feature at a frequency close to the k = 4 band position. In addition, other spectral differences exist between the live cells and membranes in the 1250-cm^{-1} region which become pronounced at high temperatures.

Figure 4 shows the temperature dependence of the CH_2 symmetric stretching frequency of pentadecanoic acid enriched cells, hydrated membrane, and the extracted membrane lipids. The thermotropic responses of the live cells and the hydrated membranes are superimposable within experimental error over the range of temperatures of cell viability, although some differences occur at elevated temperatures. In contrast, the membrane lipid extracts demonstrate quite different characteristics. The broad phase transition (25–40 °C) in the live cells and membrane extracts is replaced in the lipid extracts with a smaller magnitude (reduced change in frequency) transition between about 25–35 °C (onset temperature) and 55–60 °C (completion). Onset and completion temperatures are difficult to measure precisely because noncooperative

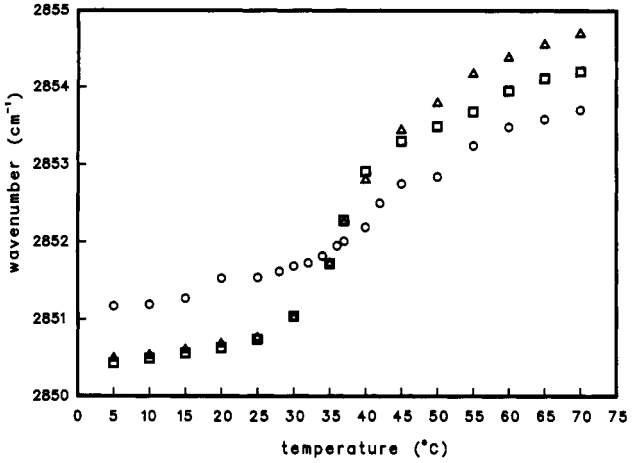

FIGURE 4: Temperature dependence of the CH_2 symmetric stretching frequency of live cells (△), isolated membrane (□), and membrane lipids (○) of C_{15}-enriched *Acholeplasma laidlawii* B as a function of temperature.

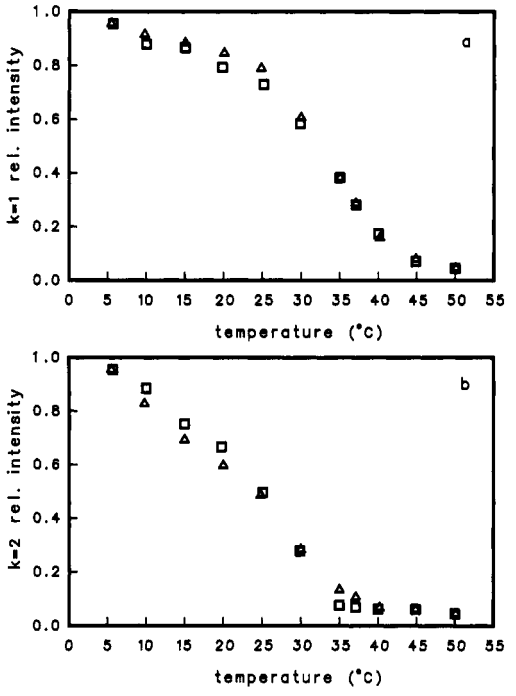

FIGURE 5: (a) $k = 1$ progression band component intensity as a function of temperature of C_{15}-enriched live cells (△) and the isolated membrane (□). (b) Equivalent data for the $k = 2$ progression band component.

changes in the frequency parameter precede and follow the more rapid rate of change that constitutes the transition. Nevertheless, it is evident that the T_m is clearly increased in the lipid extract from the other two systems. In all current experiments with pentadecanoic, myristic, or palmitic acid enriched cells, the thermotropic behavior of the live cells and the hydrated membrane was essentially the same.

To quantitatively monitor the loss of the all-trans conformation, the integrated areas of the progression bands were determined, and the intensities of the $k = 1$ and $k = 2$ bands are plotted as a function of temperature in Figure 5a,b. When the integrated peak areas for the live cells and for the hydrated membranes are scaled to coincide at the lowest temperature, as in Figure 5, the loss of intensity with temperature is virtually identical for cells and hydrated membrane. Previous studies (Senak et al., 1992) have established that the intensity of the progression monitored in this fashion is extremely sensitive to

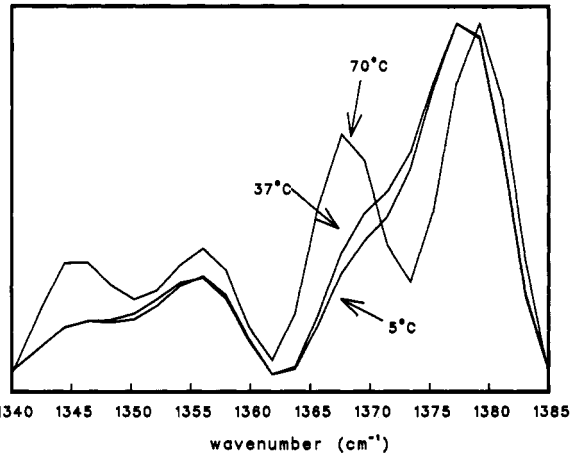

FIGURE 6: Second-derivative spectra of the extracted membrane lipids of C_{15}-enriched cells at 5, 37, and 70 °C over the 1340–1385-cm^{-1} region.

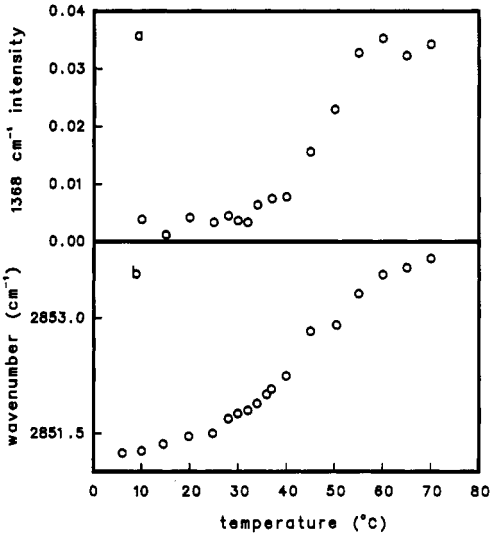

FIGURE 7: Increase in relative intensity of the 1368-cm^{-1} band as monitored for spectral subtraction (indicative of increasing kink + gtg conformations) in the C_{15}-enriched lipid extract as a function of temperature. (b) CH_2 symmetric stretching frequency as a function of temperature from the same spectra. Notice the difference in spectral sensitivity as monitored with these two structural parameters. This leads to different transition temperatures.

slight changes in conformational order. Consequently, the nearly identical thermotropic response observed in Figure 5 is a further strong indication that the live cells and membranes have the same lipid conformational states. This conclusion is *independent* of the model used to assess the extent of conformational disorder.

To examine in detail the conformational states adopted by the acyl chains of the extracted lipids during the thermotropic transition, the localized CH_2 wagging modes in the 1335–1390-cm^{-1} spectral region that characterize two- or three-bond conformers (1353 cm^{-1} for the gg state, 1340 cm^{-1} for the eg state, and 1368 cm^{-1} for the sum of kink + gtg states) in disordered phases were monitored. Figure 6 shows second-derivative spectra of the pentadecanoic acid enriched membrane lipid extract at 5, 37, and 70 °C. A substantial increase in the 1368-cm^{-1} intensity is noted as the temperature is increased. In Figure 7a, the intensity of the this band is plotted as a function of temperature. The intensity plotted is the integrated area of the 1368-cm^{-1} band in a series of difference spectra obtained by subtracting the lowest temperature spectrum from all others. A cooperative transition centered

around 50 °C is clearly evident in Figure 7a. In Figure 7b, the CH_2 symmetric stretching frequency of the lipid extract is plotted over the same temperature range. Differences in the response (hence, sensitivities of the particular parameters) of the CH_2 stretching and wagging modes are clearly evident.

The CH_2 stretching frequencies for the lipids reveal a thermotropic transition for the pure lipid phase with an onset temperature of 25–35 °C (Figure 7b). However, the frequency for this mode at low temperatures (2851.2 cm^{-1}) is characteristic of acyl chains that are already significantly disordered. The data plotted in Figure 7a emphasize changes in the relative amounts of kink + gtg forms as the temperature is raised, not the absolute concentrations of these conformational states. In contrast, the CH_2 stretching frequencies in Figure 7b are sensitive to all forms of conformational disorder in the chains and not to the particular two- and three-bond states. The transition emphasized through the change in the 1368 cm^{-1} band intensity (Figure 7a) is of unknown structural origin, since IR cannot usually be used as the definitive marker for a particular phase. It is possible, though unproven, that the observed transition may arise from the interconversion of two disordered phases such as the L_α–H_{II} transition known to occur in lipids with small polar groups. This is not an easy suggestion to test, as the physical method of choice (^{31}P NMR) is not applicable to these glycolipid samples.

The availability of synthesized diC_{16}MGDG allowed us to probe the behavior of a major lipid constituent. Spectra were collected over the temperature range −75 to +70 °C. Figure 8 shows the raw spectra at 5 and 70 °C over the 1190–1300-cm^{-1} region. The progression bands that dominate the 5 °C spectrum are completely absent at 70 °C. The lack of underlying features allowed us to determine the integrated areas of these features directly from the spectra. The intensity of the $k = 1$ component is plotted as a function of temperature in Figure 9a, while the thermotropic response of the symmetric CH_2 stretching mode is plotted in Figure 9b. The transition temperature (57–60 °C) is in excellent accord with the data of Mannock et al. (1990) for the L_β–L_α interconversion as monitored with DSC.[2]

DISCUSSION

The current experiments offer several advantages for studies of conformational order in *A. laidlawii*. First, the observation of the wagging mode progression in live cells provides strong direct evidence for the presence of substantial amounts of all-trans conformational order. The bands are unambiguously identified by comparison with model compound data such as diC_{16}MGDG and disaturated PC's (Figure 9 and Table I). Application of the semiquantitative model discussed in our previous studies (Senak et al., 1992) suggests that at the growth temperature there are about 1.7 gauche bonds/chain. This value may be compared with the presence of 3.6 gauche bonds/chain in the L_α phase of DPPC (Mendelsohn et al., 1989) and about 1 gauche bond/chain in the "liquid-ordered" state of 2/1 DPPC/cholesterol mixtures (Davies et al., 1990). Regardless of the accuracy of the model, the presence of the progression implies the existence of a substantial population of all-trans chains. Second, the nearly identical thermotropic response of the progression intensity of the live cells compared with the membrane extracts (Figure 5) provides strong evidence for the similarity of lipid acyl chain conformational order in the two cases. This conclusion is again independent of the details of the model chosen for quantitative analysis. Finally, the absence of the wagging mode progression in the lipid extracts coupled with the observation of bands arising from particular localized (disordered) two- or three-bond conformational states shows directly that the lipid extracts are substantially more conformationally disordered than either the live cells or their membrane extracts.

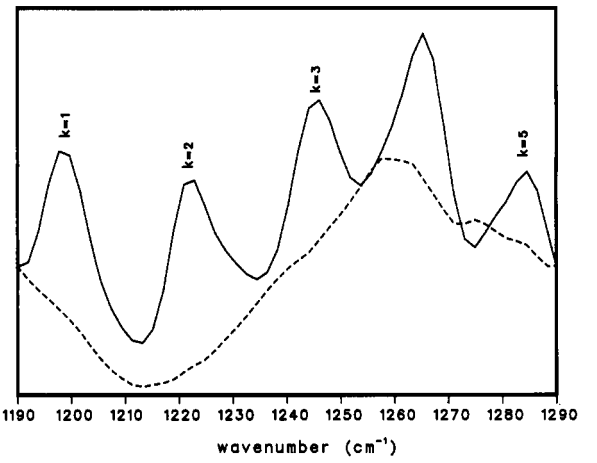

FIGURE 8: Original spectra of diC_{16}MGDG over the CH_2 wagging progression band region 1190–1290 cm^{-1} at 5 °C (—) and 70 °C (- - -).

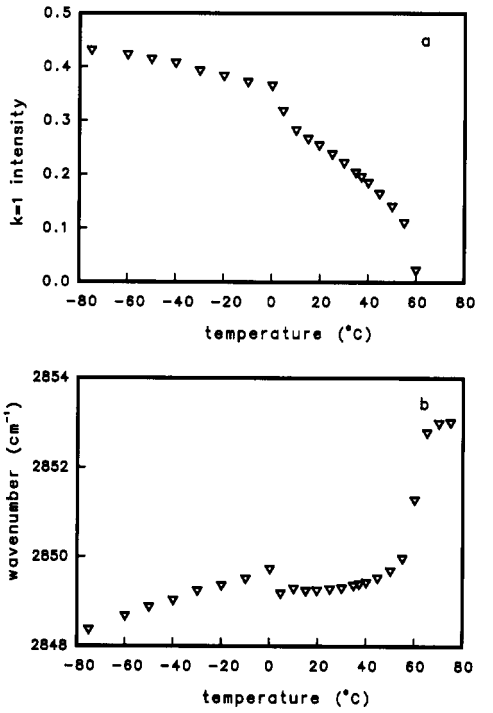

FIGURE 9: (a) Intensity of the $k = 1$ progression band component of diC_{16}MGDG from −75 °C to + 70 °C. (b) CH_2 symmetric stretching frequency of diC_{16}MGDG over the same temperature range.

The conclusions reached from studies of the wagging mode progressions are completely confirmed by comparison with the thermotropic behavior of the CH_2 stretching frequencies, an IR parameter much less amenable to quantitative interpretation. This monitor of acyl chain conformation is again virtually superimposable for the live cells and membranes (Figure 4). The frequency at low temperature (2850.4 cm^{-1}) is indicative of a relatively ordered phase and consistent with the observation of the coupled wagging mode progression. In contrast, the CH_2 symmetric stretching frequency of the lipid extract is about 0.8 cm^{-1} higher at the same temperature,

[2] A reviewer has pointed out that the discontinuities observed at about 0 °C in Figure 9 may arise from the melting of ice in the samples and the concomitant rehydration of the lipid phase.

suggestive of much more acyl chain disorder and consistent both with the lack of coupled wagging modes (indicative of all-trans chains) and with the presence of the localized wagging bands (indicative of chain disorder). A quantitative estimate of the disorder required to produce a 0.8 cm^{-1} frequency shift is available from the two-state model of Dluhy et al. (1983), which indicates that at least 50–60% disorder is required.

The current conclusions are in excellent accord with the DSC studies of Seguin et al. (1987), who demonstrated that whole cells and extracted membranes in elaidate-enriched *A. laidlawii* have identical thermotropic behavior which differs substantially from that of the extracted lipids. An advantage of the current work is that it permits elucidation of molecular structure information to complement the thermodynamic parameters extracted from DSC studies. We note that the work of Seguin et al. (1987) showed a transition enthalpy that was higher for the extracted lipids than for the intact cells. The current results (for cells containing saturated acyl chains) suggest a smaller change in the hydrocarbon chain order on melting of the isolated lipids than on melting of the whole cells or extracted membranes, which might imply a smaller enthalpy change on melting of the lipids. This suggestion must be considered unproven, as there is no established correlation between the change in the CH_2 stretching frequency in the infrared spectrum and the DSC-determined enthalpy of melting. We also note that the melting range for the lipid extracts appears to be somewhat broader (although there is an uncertainty in the assignment of onset and completion temperatures) than the intact cells or membranes.

The current conclusions are also quite compatible with the ^2H NMR measurements of Stockton et al. (1975) which claimed substantial all-trans conformational order in the extracted membranes. We note that these two methods, utilizing nonperturbing approaches, sample widely different time scales.

The current work, however, yields substantially different conclusions from the pioneering series of IR studies carried out by Mantsch and co-workers (Cameron et al., 1983, 1985; Mantsch et al., 1988). They compared *A. laidlawii* conformational order in whole cells and extracted membranes that had been biosynthetically enriched in acyl chain perdeuteriated myristic, pentadecanoic, or palmitic acids. Examination of the thermotropic behavior of the CD_2 symmetric stretching vibrations revealed substantially (and consistently) more disorder in the live cells than in the extracted membrane. The existence of two technical and procedural differences between our current work and the previous IR studies should be noted. First, the previous IR studies utilized a technique which involved the continuous acquisition of IR spectra as the temperature was continuously raised ("ramped") by 1 °C/minute from 15 to 45 °C. Thus, the whole series of spectra were collected in a period of 0.5 h. This minimal data collection time is favorable from the point of view of sample stability and viability, but it is possible that the observed differences in acyl chain conformational order between the cells and the extracted membranes as the temperature is raised occur because of different thermal lags in the two types of samples. The ramping experiments are more likely to sample nonequilibrium thermodynamic states. A similar point (emphasizing the possible occurrence of different low-temperature phases) has been made by Seguin et al. (1987). Unfortunately, our current apparatus does not permit the temperature to be ramped precisely as the IR spectra are generated. We therefore, cannot test this suggestion.

The current experiments were carried out under conditions where all samples were equilibrated for 2 min at each temperature. To minimize IR sampling time, current temperature intervals were 2 or 3 °C i.e., significantly greater than the prior experiments. Sample to sample variation in the current experiments was minimal. Complete replicate runs (duplicate cultures and IR protocols) produced essentially superimposable thermotropic parameters.

The other major difference between the studies is that the current work utilizes fully proteated fatty acids to permit observation of the coupled CH_2 wagging progression intensity, while perdeuteriated fatty acids in the prior work permitted the isolation of CD_2 stretching bands. It is improbable that the comparative thermotropic behavior of cells vs membranes would be subject to a large isotope effect. In any case, many IR studies of phosphatidylcholines have utilized CD_2 or CH_2 stretching modes to qualitatively track conformational order (Mendelsohn & Mantsch, 1986), without any hint of major isotopic differences, except for a small decrease (4–5 °C upon acyl chain perdeuteriation of DPPC) in T_m.

An advantage of the current approach is that the CH_2 wagging vibrations are direct indicators of either all-trans conformational order (the progression band series) or chain disorder arising from particular two- or three-bond conformational states (the localized modes). Intensities of the latter can be calibrated via theories of the alkane liquid state, such as the rotational isomeric state model (Flory, 1969) used to generate reference state populations. The ability of current IR spectrometers coupled with modern data analysis protocols to detect the wagging bands suggests immediate extensions of the current experiments in a variety of directions of interest in cell biology.

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