Adaptation to Altered Growth Temperatures in *Acholeplasma laidlawii* B: Fourier Transform Infrared Studies of Acyl Chain Conformational Order in Live Cells†

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ABSTRACT: *Acholeplasma laidlawii* B cells, highly enriched in saturated C_{14} and C_{15} fatty acids, have been grown at several temperatures. Conformational order in the acyl chains of live cell membranes has been monitored with Fourier-transform infrared (FT-IR) spectroscopy. Coupled CH_2 wagging progressions, characteristic of the all-trans conformation, have been used to quantitatively monitor the extent of trans–gauche isomerization. A simple model relating the changes in the progression intensity to the introduction of gauche rotamers into the acyl chains suggests that at the growth temperature (25 or 37 °C for the C_{14}-enriched cells, 30 or 37 °C for the C_{15}-enriched cells) the cell membrane contains about 1.5 gauche bonds per acyl chain. The thermotropic response of the CH_2 stretching frequencies near 2850 cm^{-1} reveals that the gel–liquid-crystal phase transition is shifted toward lower temperatures when the growth temperature is reduced. In addition, at any given temperature, the cells grown at lower temperature are more conformationally disordered than their counterparts grown at higher temperature. This behavior is consistent with the quantitative results from the CH_2 wagging measurements. The data reveal that *A. laidlawii* B cells can control the overall conformational state of their membranes and that the observed degree of disorder (1.5 ± 0.3 gauche bonds/acyl chain), when achievable, provides optimum cell viability. The ability of this microorganism to control the degree of membrane disorder delineates one possible pathway for homeoviscous adaptation.

Molecular structure information is sparce concerning the *in situ* response of the plasma membranes in living cells to environmental changes. Advances in this area require the combination of a system whose membrane composition can be controlled to a substantial degree, along with a suitable physical method which can yield reliable conformational or dynamic information from systems as complex as whole cells. The ability of the cell wall-less procaryote *Acholeplasma laidlawii* to adjust the physical and chemical properties of its plasma membrane in response to altered growth temperature has been reviewed (McElhaney, 1984, 1989). This microorganism possesses several particular advantages for biochemical and biophysical investigations of membrane structure. First, the lack of a cell wall and the absence of organelles facilitate plasma membrane isolation and characterization. Second, a wide variety of exogenous fatty acids may be incorporated to near-homogeneity in the membrane acyl chains, thereby simplyifying the analysis of biophysical experiments. Finally, the live cells undergo a thermotropic event resembling the bilayer gel → liquid-crystal phase transition of isolated lipids, thereby rendering relevant the extensive structural and dynamic information available from studies of model compounds (Melchior et al., 1970; Stein et al., 1969; Seguin et al., 1987).

Biochemical studies have revealed changes in the ratio of the neutral glycolipids monoglycosyldiacylglycerol (MGDG),[1] a hexagonal phase preferring lipid to DGDG, a bilayer phase preferring lipid, in the plasma membrane of *A. laidlawii* strain A as a response to altered growth temperature. A variety of physical factors have been suggested as being needed to ensure optimal membrane function (Lindblom & Rilfors, 1992; Rilfors et al., 1993; Bhakoo & McElhaney, 1988).

In addition to the biochemical studies, the biophysical approaches of DSC, ESR spectroscopy, and DPH fluorescence have been used to monitor changes in cell membrane thermodynamics and fluidity as a response to altered growth conditions. Inconsistencies in conclusions drawn from probe-requiring spectroscopic experiments (ESR and DPH fluorescence) have recently been discussed (McElhaney, 1993). Evidently, probe-induced perturbations of membrane properties and ambiguities about the location of the probe in the membrane lead to difficulties in extrapolation from the measured spectroscopic properties of the probe to accurate conclusions about putative changes in membrane properties upon adaptation. DSC has provided complete thermodynamic characterization of lipid phase transitions in *A. laidlawii*, but yields no direct molecular structure information about the individual phases sampled.

The advantages of FT-IR spectroscopy for studies of membrane conformational order in model systems and living cells are well documented (Mendelsohn & Mantsch, 1986; Mendelsohn & Senak, 1993; Moore et al., 1993; Cameron et al., 1985). The technique does not require probe molecules, utilizes small sample volumes, provides information about localized regions within the lipid molecules, and yields quantitative information about conformational order in the membrane acyl chains. For example, this laboratory recently demonstrated that the coupled CH_2 wagging vibrations, which

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[1] Abbreviations: DGDG, diglucosyldiacylglycerol; DPPC, 1,2-dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; DPH, diphenylhexatriene; FT-IR, Fourier transform infrared; ESR, electron spin resonance; MGDG, monoglucosyldiacylglycerol; TLC, thin-layer chromatography.

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quantitatively probe acyl chain trans–gauche isomerization in relatively ordered lipid phases, can be detected in live cells of *A. laidlawii* B. Conformational order in living cells is virtually identical to that in extracted membranes but differs widely from the isolated lipids (Moore et al., 1993). The current study describes an FT-IR study of the effects of altered growth temperature on membrane conformational order in live cells of *A. laidlawii* B enriched in saturated C_{14} or C_{15} acyl chains. These experiments suggest a partial basis for the phenomenon of homeoviscous adaptation.

MATERIALS AND METHODS

Cell Growth. All procedures have recently been described in detail (Moore et al., 1993). Briefly, *Acholeplasma laidlawii* B cells were grown in a lipid-depleted medium in the presence of avidin (Silvius & McElhaney, 1978). Saturated C_{14} or C_{15} fatty acids were added to the growth media as concentrated ethanolic solutions. Cells enriched with C_{14} were grown at 25 and 37 °C while cells enriched in C_{15} were grown at 30 and 37 °C and were harvested at the late log phase by centrifugation. Cells were checked for viability as described.

Membrane Preparation and Analysis. For membrane isolation, the cells were lysed by osmotic shock. Those membrane samples not used immediately were lyophilized and rehydrated as required. For analysis of membrane acyl chain composition, the methyl esters of the membrane lipids were prepared and analyzed by gas chromatography. Homogeneity (as measured by the proportion of the sought acyl chain length) was routinely above 92% for C_{15}-enriched cell membranes grown at 37 and 30 °C; we were unable to grow cells with exogenous pentadecanoic acid at 25 °C. There was more variation in the C_{14}-enriched cells; cells grown at 37 °C reached over 90% homogeneity in the acyl chain composition, while the highest level attained in cells grown at 25 °C was 88%. In all cases, only cells with homogeneity levels above 70% were used for analysis. The head-group composition, as deduced from TLC, revealed the lipid main classes expected (McElhaney, 1984) for *A. laidlawii*; these included the neutral glycolipids MGDG and DGDG, the phospholipid phosphatidylglycerol, and the phosphoglycolipid glycerophosphoryldiglucosyldiacylglycerol. Although variations in the relative proportions of these were noted from sample to sample, no systematic patterns could be discerned, consistent with previous observations for *A. laidlawii* B (Bhakoo & McElhaney, 1988).

FT-IR Methods. Samples for IR spectroscopy were prepared by centrifugation and then placed between two CaF_2 windows separated by a 12-μm spacer. FT-IR spectra were collected on a Mattson Instruments Research Series (RS-1) spectrometer equipped with a mercury cadmium telluride 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). For experiments at temperatures below 0 °C, spectra were acquired in a liquid N_2 cooled dewar. Temperature was regulated by the controlled boil-off of liquid N_2 and monitored with a thermocouple. 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.

FT-IR Data Analysis. Spectra were collected from 5 to 70 °C at 3–5 °C intervals. Samples were allowed to equilibrate for several minutes at each temperature prior to data acquisition. Quantitative analysis of the CH_2 coupled wagging modes was accomplished as follows: A spectrum obtained at 70 °C for a given preparation was subtracted over the 1190–1290 cm^{-1} spectral region from spectra acquired at lower temperatures. The difference spectra, generated to produce consistent background shapes, were integrated between minima to give the intensity of the individual progression band components. The $k = 1$ component of the progression bands was routinely selected as the indicator of acyl chain conformation. The presence of shifting underlying modes (due possibly to phosphate PO_2^- asymmetric stretch and protein amide III) in the 1220–1260 cm^{-1} region overlapped the $k = 2$ and $k = 3$ modes and rendered them unsuitable for quantitation. To determine the frequency of the CH_2 symmetric stretching mode, a spectrum of water, matched for path length and temperature, was subtracted from each spectrum over the 2800–3000 cm^{-1} region. The frequencies of the CH_2 stretching modes were then calculated with a center of gravity algorithm to give band positions with an uncertainty of less than 0.1 cm^{-1} (Cameron & Moffatt, 1984).

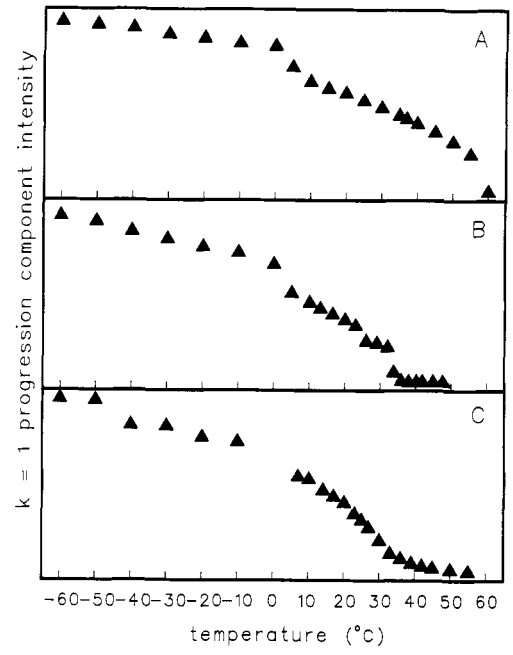

FIGURE 1: $k = 1$ progression band component intensity as a function of temperature of (A) diC_{16}-MGDG, (B) diC_{15}-PC, and (C) C_{15}-enriched cell membranes.

Calculations. To estimate from the CH_2 wagging progression intensities the amount of disorder present in the acyl chains at the growth temperature, it is necessary to determine as a reference state the progression intensity when all acyl chains are in the all-trans conformation. Experiments shown in Figure 1 suggest that at ≈–60 °C, the progression bands reach their maximum intensity, indicating that the vast majority of chains are in the all-trans conformation. Figure 1A and Figure 1B show data for the model lipids diC_{16}-MGDG and diC_{15}-PC respectively, whereas the results from the spectra of whole C_{15}-enriched cell membranes are plotted in Figure 1C. In Figure 1A,B, the progression intensity vanishes at T_m for the lipid, consistent with previous work (Moore et al., 1993). The intensity of the CH_2 wagging progression in each case appears to reach an upper limit at –60 °C, thus suggesting a fully ordered state. Further evidence for the all-trans form at low temperature comes from the work of Cameron and co-workers, who monitored the splitting of the CH_2 scissoring band (1460 cm^{-1}) of DPPC. They found that a limiting value

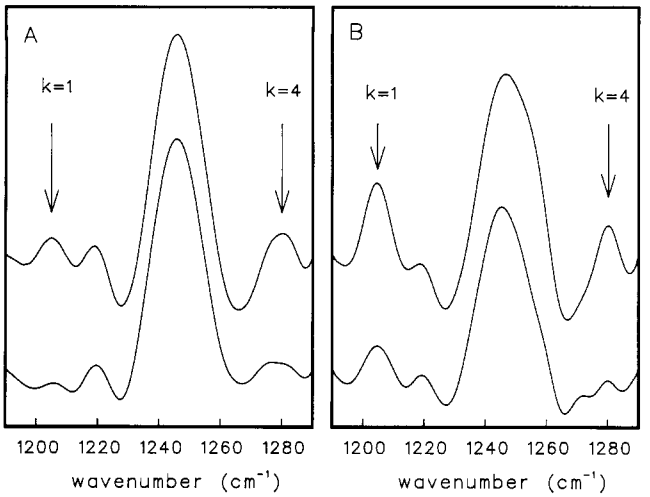

FIGURE 2: (A) Difference spectra (resulting from subtraction of the 70 °C spectrum) at 25 °C (upper spectrum) and 37 °C of C_{14}-enriched cells grown at 25 °C. (B) The equivalent difference spectra of C_{14}-enriched cells grown at 37 °C.

Table 1: CH_2 Wagging Mode Progression Frequencies (cm^{-1}) in Live Cells

progression component	$k = 1$	$k = 4$
diC_{14}-PC	1203.7	1280.8
C_{14} cells (grown at 25 °C)	1204.2	1280.7
C_{14} cells (grown at 37 °C)	1204.3	1280.4
diC_{15}-PC	1201.7	1273.1
C_{15} cells (grown at 30 °C)	1202.0	1273.4
C_{15} cells (grown at 37 °C)	1201.8	1272.8

of the splitting was achieved at ≈–50 °C, implying cessation of chain rotational motion and, presumably, complete chain conformational order (Cameron et al., 1980).

RESULTS

Figure 2 shows typical difference spectra in the CH_2 wagging region at 25 and 37 °C for C_{14}-enriched cells grown at 25 °C (Figure 2A) and 37 °C (Figure 2B). The progression components are labeled in the figure. A reduced $k = 1$ intensity in spectra taken at 25 and 37 °C is clearly evident for the cells grown at 25 °C when compared (with data at these temperatures) to cells grown at 37 °C. The spectra reveal that the cells are able to regulate their membrane conformational order in response to the growth temperature and produce a more ordered membrane at higher temperatures. This conclusion is independent of the model used to convert the measured difference spectral intensities to probabilities of the all-trans conformation.

The frequencies of the $k = 1$ and $k = 4$ components were determined for both C_{14}- and C_{15}-enriched cell cultures at all growth temperatures. Table 1 lists these frequencies along with model C_{14} and C_{15} lipids. The agreement between the model lipids and live cells confirms the assignment of progression band components.

The intensity of the $k = 1$ component of C_{14}-enriched cells from spectra of *A. laidlawii* grown at 25 and at 37 °C is plotted as a function of temperature in Figure 3. Each growth temperature is marked on the appropriate curve. The data clearly show that the thermotropic response of the coupled CH_2 wagging modes in the two systems is widely different. In addition, the cell adapts such that at the growth temperature the degree of order is the same within experimental error for each growth temperature. According to the quantitative analysis, the conformational disorder at the growth temperature corresponds to about 1.5 gauche bonds per acyl chain. Table 2 lists the calculated means and estimated errors of all experiments and demonstrates the extremely good agreement among the various data sets. In Figure 4, the data for the $k = 1$ progression intensities are presented for pentadecaonic acid-enriched cells grown at 30 and 37 °C. Adaptation in response to changes in growth temperature is again evident from measurement of the CH_2 wagging progression intensities. The extent of disorder is summarized in Table 2.

The thermotropic behavior of the CH_2 symmetric stretching frequency of C_{14}-enriched cells grown at 25 and 37 °C is plotted in Figure 5. In Figure 6 the equivalent data are plotted for cells enriched in C_{15} grown at 30 and 37 °C. The growth temperatures are indicated on each data set. A shift to higher temperature in the phase transition of the lipid from gel

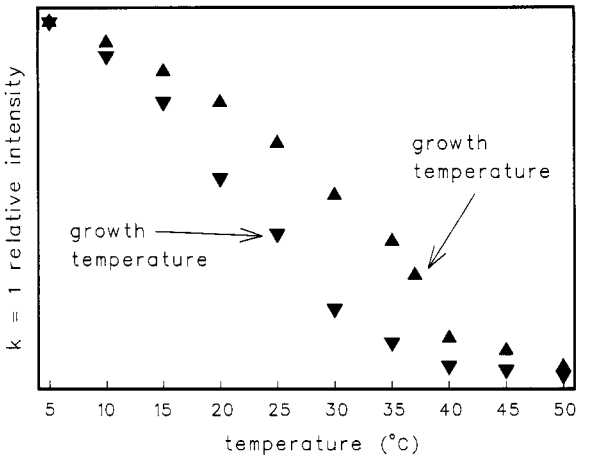

FIGURE 3: $k = 1$ component relative intensity plotted as a function of temperature for C_{14}-enriched cells grown at 25 °C (▼) and 37 °C (▲).

Table 2: Number of Gauche Rotamers per Acyl Chain in Live Cells at the Indicated Growth Temperature

	25 °C	30 °C	37 °C
C_{14}-enriched cells[a]	1.3 ± 0.2		1.6 ± 0.2
C_{15}-enriched cells[b]		1.4 ± 0.2	1.3 ± 0.2

[a] Mean of five separate cell incubation and spectroscopy experiments each at 25 and 37 °C. Error estimate incorporates the uncertainty in the intensity of the progression in the –60 °C reference state. [b] Mean of three separate cell incubation and spectroscopy experiments each at 30 and 37 °C. Error estimates incorporate the uncertainty in the intensity of the progression in the –60 °C reference state.

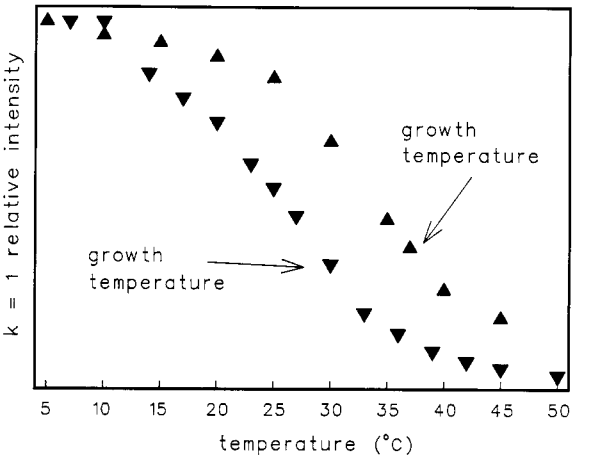

FIGURE 4: $k = 1$ component relative intensity plotted as a function of temperature for C_{15}-enriched cells grown at 30 °C (▼) and 37 °C (▲).

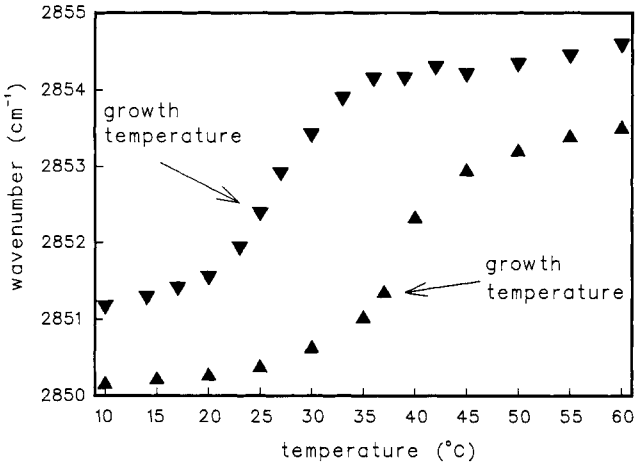

FIGURE 5: Temperature dependence of the CH_2 symmetric stretching frequency of C_{14}-enriched cells grown at 25 °C (▼) and 37 °C (▲). Note that the absolute values of the stretching frequency are higher for cells grown at 25 °C, and the T_m is shifted to lower temperature.

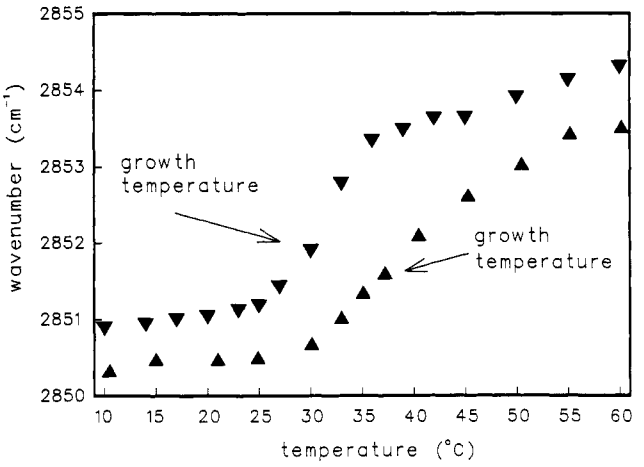

FIGURE 6: Temperature dependence of the CH_2 symmetric stretching frequency of C_{15}-enriched cells grown at 30 °C (▼) and 37 °C (▲). Again the frequency values are higher for cells grown at 30 °C, and the T_m is shifted to lower temperature.

to liquid-crystal is evident in cells grown at 37 °C. In all cases, the observed midpoint in frequency vs temperature data is slightly higher than the growth temperature. The frequency of the CH_2 symmetric stretch is higher at all temperatures in the cells grown at the lower temperature in both the C_{14} and C_{15} systems. Since the frequency of the CH_2 stretching modes increases upon introduction of conformational disorder in the acyl chains, these data suggest there is more disorder in the cell membranes at the lower growth temperatures. To quantitatively evaluate this suggestion, the number of gauche rotamers was evaluated over a range of temperatures from the CH_2 wagging progressions. The data are plotted in Figure 7 and provide quantitative support for the CH_2 stretching frequency data. The membranes of cells grown at lower temperature are more disordered at all temperatures than the membranes of cell grown at 37 °C.

DISCUSSION

A significant advantage of the current experimental approach is the availability of precise, *in situ*, conformational information from the membranes of living cells. The CH_2 wagging progression directly monitors trans–gauche isomerization in the acyl chains, and hence provides a "snapshot" of their conformational state. Such data have heretofore been unavailable from biophysical measurements of intact cells.

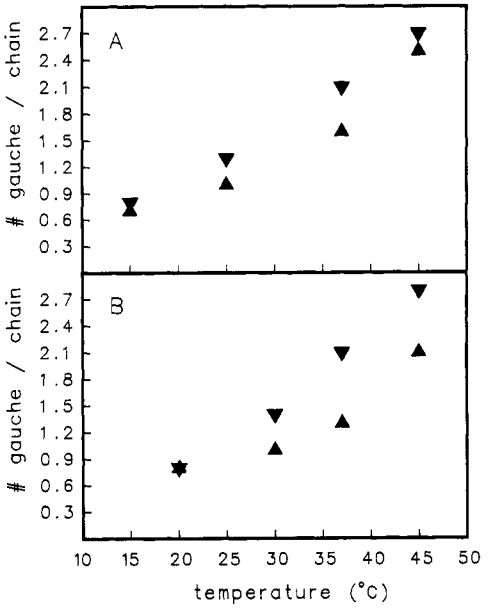

FIGURE 7: (A) Calculated number of gauche rotamers per chain as a function of temperature in C_{14}-enriched cells grown at 25 °C (▼) and 37 °C (▲). (B) The equivalent data for C_{15}-enriched cells grown at 30 °C (▼) and 37 °C (▲). Note that the cells grown at lower temperature are more disordered at all temperatures than cells grown at 37 °C.

The marked adaptation of acyl chain conformation to alterations in the growth temperature is clearly evident from the data in Figures 2–7, both in terms of the actual conformational state of the chains at the various growth temperatures, and in terms of the thermotropic response of the cells. It is relevant to note that both the CH_2 wagging progression intensity and the CH_2 stretching frequency are nonlinear functions of the extent of gauche rotamer formation. The model for the variation of the wagging progression intensity as described in the Appendix predicts that a small fraction of conformational disorder produces a large intensity diminution. For example, a 20% probability of gauche bond formation at each chain position (similar to that observed at several acyl chain positions of L_α phase DPPC) produces a relative progression intensity of 5%. The latter value is probably close to our detection limit, so that maximally disordered phases produce little CH_2 wagging progression intensity, and hence are not easily monitored. This spectral parameter is most useful for sampling the conformation of phases that are slightly disordered. The detection of progression intensity in the plasma membrane of living cells is consistent with a relatively ordered (compared with the L_α) phase present at the growth temperature.

The current studies show that, under the limited set of growth temperatures and fatty acids sampled, *A. laidlawii* B is able to adapt so that at the growth temperature a conformational state containing about 1.5 gauche bonds per chain is present. Relevant comparisons as to levels of conformational disorder are available from earlier IR investigations of model membrane systems. The CD_2 rocking modes of specifically deuterated DPPC reveal that the L_α phase possesses 3.6 gauche bonds per chain (Mendelsohn et al., 1989). Recent molecular modeling calculations (De Loof et al., 1991) are in accord with this experimental result, which engenders some confidence in both the experimental and theoretical methods employed. Adjusting for the shorter chain lengths in the current work, an appropriate comparison would be 3.3 and 3.1 gauche bonds/chain for the L_α states of 1,2-dipentadecanoyl-PC and 1,2-DMPC, respectively. The effect of varying the lipid class on

the level of quantitative conformational disorder (in mixtures of MGDG and DGDG, for example) is not known, so that head-group-induced differences in acyl chain conformational order between the PC model systems and the MGDG and DGDG species that dominate the *A. laidlawii* membrane cannot be evaluated.

Addition of 33 mol % cholesterol to DPPC produces the positionally disordered but conformationally ordered "liquid-ordered" phase (Vist & Davis, 1990; Ipsen et al., 1989). Conformational disorder in this state decreases from 3.6 gauche bonds per chain in pure DPPC to about 1.5 (Davies et al., 1990). Whether the similarity in conformational disorder between the biologically relevant (for eucaryotic plasma membranes) "liquid-ordered" phase and the values achieved at the *A. laidlawii* growth temperature in the current work is of general significance, or is merely an accident of the chosen fatty acids, must await further study.

The second parameter utilized to monitor conformational order is the acyl chain CH_2 symmetric stretching frequency. As note above, variation in this parameter is a highly nonlinear function of conformational disorder. A simple two-state model (for a near-ideal two-component system) for the amount of disordered phase required to induce a particular variation in the frequency shows that to produce 50% of the change in the measured frequency parameter, the "fraction of disordered lipid" needed is about 0.8 (Dluhy et al., 1983). The nature of the disordered state is unspecified. In the current melting profiles (Figures 5 and 6), the observed CH_2 stretching frequencies at the growth temperature are much closer to the order-state value rather than the disordered-state value. This observation suggests a limited amount of conformational disorder, consistent with the detection of intensity in the wagging progression.

A limitation of the current approach is the unknown accuracy of the model which connects the measured intensities with gauche probabilities in the acyl chains. The underlying assumption is that a dynamic gauch rotation rapidly changing sites along the chain would completely destroy the sharp component bands of the wagging progression. A gauche rotation at a particular chain location would be expected to uncouple (at least partially) the two resulting segments of the chain to produce frequency patterns characteristic of the number of methylenes in the segment closest to the polar head group (Chia & Mendelsohn, 1992). The sum of these patterns (from a series of segment lengths) might then produce an overlapped band contour contributing to the measured spectral intensity. However, our data reduction protocols (subtraction of a high-temperature spectrum) would tend to eliminate a substantial portion of broad underlying contributions to the sharp progression bands.

Generalization of the current results from the limited set of experiments is risky; nevertheless, it appears that, when achievable, a preferred state of the lipid acyl chains is substantially more ordered than the L_α liquid-crystalline phase. A limitation of the current experiments is that cell growth with exogenous saturated fatty acids occurs over a limited range of temperatures. Nevertheless, a 12 °C growth temperature difference in the C_{14}-enriched membranes resulted in essentially the same degree of conformational order at the two growth temperatures. This result suggests the presence of mechanisms for regulation of conformational order in the membrane. Obviously, such mechanisms are not the sole means by which the adaptation process operates. For example, McElhaney has shown that the absolute minimum growth temperature for *A. laidlawii* is 8 °C, a value which can be achieved with exogenous unsaturated fatty acids (McElhaney, 1974). Since growth under these conditions is optimum at temperatures well over 30 °C, it is unlikely that cells grown over this wide a temperature interval could possess significant acyl chain conformational order at the higher end of the growth temperature range. Thus, other regulation mechanisms are probably operational. Additional factors such as protein content and lipid class could be altered or controlled in response to changes in the growth environment. These ideas are currently under investigation with the FT-IR technology described here.

APPENDIX

A Semiquantitative Model for Determination of Conformational Order from Wagging Progression Intensities. Several assumptions/approximations are required to convert the wagging progression intensities into a measure of acyl chain conformational order. First, it is assumed that the intensity is derived solely from all-trans chains. This demands that all other configurations (including states containing a gauche bond near the head group or near the bilayer center) destroy the coupling that produces the progression. In the absence of reliable IR intensity calculations for model phospholipids in particular disordered states (currently unavailable), it is impossible to know if any fraction of the progression intensity "leaks" through a gauche bond. A large disruption in the regularity of an all-trans chain, such as induced by a cis C=C bond, *is* in fact known (Chia & Mendelsohn, 1992) to completely destroy the coupling between the two resulting sets of methylenes. Intensity calculations are complicated by the fact that the progression intensity is mostly determined by the dipole moment change of the ester C–O bond.

Although we showed (Figure 1) that full conformational order is probably achieved at –60 °C, live cells could not be conveniently examined by IR at low temperatures and carried through viably to the growth temperature. It was thus necessary to introduce a numerical factor, obtained from the data in Figure 1, that correlated the progression intensity from its measured value at the lowest convenient experimental temperature for whole cells (5 °C) to its value at –60 °C when the chains are fully all-trans. As shown in Figure 1, the intensity of the progression band components increases by a factor of about 1.5–2 from 5 to –60 °C. According to the model below, the increase in the progression intensity when the temperature is lowered is induced by a small increase in conformational order at a given chain position. For example, an assumed value of 2 for I_{-60}/I_5 (where the I's are the intensities at the subscripted temperatures) corresponds to a 5% probability of a gauche rotamer being formed at any C–C bond in a C_{15} lipid acyl chain ($\sim$0.6 gauche bond in a C_{15} chain) at 5 °C. The probability is quite insensitive to the correction factor chosen. Thus, if I_{-60}/I_5 is 1.5 instead of 2, the gauche probability at 5 °C is calculated to be 3%, equivalent to $\sim$0.4 gauche bond in a C_{15} chain. The uncertainty induced in the conformational disorder by our choice of correction factor is included in our error estimates.

With the above as a basis, we have constructed a simple model for introduction of conformational disorder. The integrated intensity of the $k = 1$ mode at 5 °C is multiplied by 2 (the assumed correction factor) to produce the intensity of the reference state (all-trans conformation). At temperatures above –60 °C, the probability of a trans bond is p_t, where p_t is close to, but less than, unity. The relative intensity of the progression is

$$I_t/I_{-60} = (p_t)^n$$

where I_t is the intensity at a temperature t and n is the number of methylenes. The number of gauche bonds in a chain is given by $(n - 1)(1 - p_t)$. It is noted that the progression intensities for the C_{15}-containing species cannot be directly compared with those of C_{14}-containing species.

There is some justification that the aforementioned assumptions underlying our analysis are useful approximations. In previous studies from this laboratory, we compared phospholipid acyl chain order in the "liquid-ordered" phase known to occur for DPPC/cholesterol mixtures, as deduced from two distinct types of IR experiments. First (Mendelsohn et al., 1991) we used the CD_2 rocking modes of specifically deuterated DPPC, whose intensities are correlated directly with gauche bond occurrence, to show that about 1.2 gauche bonds/chain occur at 50 °C. This compared reasonably well with a value of 1–1.5 gauche bonds/chain deduced from the wagging progression intensities for the same system (Chia et al., 1993). The primary motivation for the current overall approach is the enormous technical difficulty in detecting the CD_2 rocking modes in living cells.

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