1982). It may now be possible to conduct studies with the oligomers to eliminate the charge repulsion that occurs between the auto-poly(ADP-ribosylated) enzyme and other portions of the DNA strand not essential for enzyme activation.

The results of this study also suggest that caution should be taken in interpreting some results of enzyme activation at the cellular level. It has recently been reported that certain metabolic or solvent perturbations increase the activity of poly(ADP-ribose) polymerase without changing the number of DNA strand breaks (Juares-Salinas et al., 1984). Such increases in activity are probably due to modulation of enzyme molecules that are already activated by existing strand breaks. However, the possibility must now be considered that very small DNA fragments, difficult to detect by standard methods, may become associated with the enzyme and involved in its activation.

Registry No. d(AGCT), 84520-45-6; d(GTTAAC), 94052-52-5; d(GGAATTCC), 70755-49-6; d(CCGAATTCGG), 63734-81-6; d(CCCGAATTCGGG), 96492-36-3; poly(ADP-ribose) polymerase, 9055-67-8.

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Infrared Spectroscopic Study of the Gel to Liquid-Crystal Phase Transition in Live *Acholeplasma laidlawii* Cells†

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ABSTRACT: The temperature dependences of the infrared spectra of deuterium-labeled plasma membranes of live *Acholeplasma laidlawii* B cells and of the isolated plasma membranes demonstrate that the profiles of the gel to liquid-crystal phase transitions are very different. At temperatures within the range of the phase transition, the live mycoplasma is able to keep the "fluidity" of its plasma membrane at a much higher value than that of the isolated plasma membrane at the same temperature. The difference is particularly pronounced at and around the temperature of growth. Live *Acholeplasma laidlawii*, grown at 37 °C on a fatty acid depleted medium supplemented with myristic acid (C14:0), pentadecanoic acid (C15:0), or palmitic acid (C16:0), are highly "fluid"; i.e., at the temperature of growth, the fractional population of the liquid-crystalline phase is 95–100% at 37 °C, whereas in the case of the isolated plasma membranes the fractional population of the liquid-crystalline phase at 37 °C is only 58% (C14:0), 36% (C15:0), or 38% (C16:0).

Few biological membranes have been more thoroughly studied to date than the plasma membrane of the microorganism *Acholeplasma laidlawii* (*A. laidlawii*).[1] The procaryotic *Acholeplasma laidlawii* belongs to the subbacterial family of mycoplasmas, the simplest microorganisms capable of autonomous growth and reproduction in cell-free media (Razin, 1979, 1982). *A. laidlawii* cells have no cell wall and possess only a single membrane system, the limiting or plasma membrane, which contains practically all the cellular lipid and a large fraction of the cellular protein as well. The plasma membrane can be easily isolated from the rest of the cell content by mild osmotic lysis and washing procedures. Furthermore, *A. laidlawii* cells readily incorporate exogenous fatty acids (including deuterated fatty acids) into the endogenous lipid pool of the plasma membrane (Silvius & McElhaney, 1978; Silvius et al., 1980; Jarrell et al., 1982). If the protein avidin is added to the growth medium, the de novo fatty acid

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[1] Abbreviations: C14:0-d_{27}, perdeuteriomyristic acid; C15:0-d_{29}, perdeuteriopentadecanoic acid; C16:0-d_{31}, perdeuteriopalmitic acid; PG, phosphatidylglycerol; MGDG, monoglucosyl diglyceride; DGDG, diglucosyl diglyceride; *A. laidlawii*, *Acholeplasma laidlawii* B; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; IR, infrared; TLC, thin-layer chromatography.

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mechanism is rendered inoperable, which leads to plasma membranes whose lipids are highly (up to 98%) enriched in the exogenous fatty acid. Thus, this organism is ideally suited for in vivo and in vitro membrane studies by physical techniques under well-defined and controllable conditions.

A characteristic of *A. laidlawii* membranes is that they exhibit a transition from a conformationally ordered low-temperature phase to a conformationally disordered high-temperature phase (McElhaney, 1984a; Smith, 1984). For membranes obtained from *A. laidlawii* cells grown on unsaturated fatty acids, this transition generally occurs at temperatures well below the growth temperature; however, if the microorganism is supplied with a saturated normal fatty acid of length 14–16 carbons, the transition occurs at temperatures close to the growth temperature. The membranes isolated from *A. laidlawii* cells grown on one of these fatty acids in the presence of avidin contain lipids with essentially only a single fatty acyl group. Consequently, they exhibit very well-defined phase transitions which bear a striking resemblance to the gel to liquid-crystal phase transitions of lipid model membranes [for a recent review, see McElhaney (1984b)].

On the other hand, we have recently found evidence that the phase transition of membranes in *A. laidlawii* cells is affected by the process of membrane isolation (Cameron et al., 1983). Thus, the phase transition occurred at a different (i.e., lower) temperature for live *A. laidlawii* cells grown at 30 °C on myristic acid compared to that observed for the isolated membranes. In order to confirm this earlier observation, we have now performed a critical comparison between the thermotropic phase behavior of isolated plasma membranes and that of plasma membranes of live *A. laidlawii* cells grown at a different temperature (i.e., 37 °C) and on different deuterated fatty acids. Our method of choice for these in vivo studies was Fourier-transform infrared spectroscopy.

Experimental Procedures

Materials. Acholeplasma laidlawii B cells were grown at 37 °C in a lipid-depleted bovine serum albumin/tryptose medium, adjusted to pH 8.1. The 5 L of growth medium contained the protein avidin (25 units/L, Sigma grade II) and was supplemented with either 0.078 mM perdeuteriomyristic acid (Merck Sharp & Dohme, 98 atom % deuterium), 0.074 mM perdeuteriopentadecanoic acid (U.S. Biochemical Corp., 95 atom % deuterium), or 0.069 mM perdeuteriopalmitic acid (Merck Sharp & Dohme, 98 atom % deuterium). After 24 h, the cells were harvested by centrifugation (9000 rpm) and washed twice with 25 mM Hepes buffer containing 20% sucrose. These concentrated suspensions of *A. laidlawii cells* were used as such for the IR spectroscopic experiment. As soon as the IR experiment was finished (approximately 25 min), the *A. laidlawii* cells were examined for lysis and recultured in the same growth medium as well as on an agar gel at 37 °C; both cultures were successful and indicated that >95% of the cells were viable.

Freshly prepared plasma membranes were obtained by osmotic lysis, washed 3 times with deionized water, concentrated by centrifugation (18 000 rpm), and used in the IR experiment without the process of freeze-drying and rehydration.

For acyl chain analysis, the membrane lipids were hydrolyzed and methylated in 0.7 N methanolic HCl and extracted with *n*-hexane. The methyl esters of the fatty acids were analyzed on a Varian Model 3700 gas chromatograph equipped with a direct injector using a 50 m × 0.25 mm i.d. fused silica column (Quadrex Corp.) with helium as carrier gas. The data were analyzed with a Varian VISTA 401 data system. Heptadecanoic acid was used as the internal standard. The degree of incorporation of the exogenous deuterated fatty acids into the membranes of *A. laidlawii* is shown in Table I.

Table I: Lipid Composition and Fraction of Liquid-Crystalline Phase Present in *Acholeplasma laidlawii* Membranes at the Temperature of Growth

exogenous fatty acid	degree of incorpn (%)	MGDG/DGDG ratio	fractional population (%) of membrane lipids in liquid-crystalline phase at 37 °C	
			live cells	isolated membranes
C14:0-d_{27}	86	0.32	100	60
C15:0-d_{29}	98	2.7	96	36
C16:0-d_{31}	71	5.25	99	38

The extraction of membrane lipids was performed according to the method of Bligh & Dyer (1959). About 20 mg of membranes was suspended in 2 mL of H_2O, and then 5 mL of CH_3OH and 2.5 mL of $CHCl_3$ were added. The mixture was stirred for 6 h at room temperature; 1 mL of H_2O and 1 mL of $CHCl_3$ were added, and then the mixture was centrifuged for 10 min at 4000 rpm. The chloroform layer was dried over Na_2SO_4, filtered off, and concentrated to about 1 mL under a stream of nitrogen; this chloroform extract was applied onto precoated TLC plates (silica gel 60, layer thickness 0.25 mm) and eluted with $CHCl_3/CH_3OH/H_2O$ (64:25:4). Short exposure to iodine vapor revealed seven spots which were identified (in decreasing polarity) as phosphatidylglycerol *o*-amino esters, cardiolipin (diphosphatidylglycerol), phosphatidylglycerol, diglucosyl diglyceride, monoglucosyl diglyceride, and a carotenoid pigment (Wieslander & Rilfors, 1977; McElhaney, 1984b). The individual lipid zones were scraped off the TLC plates, transmethylated, and analyzed for acyl chain composition as described above.

Methods. Samples (about 20 mg of the suspended live cells or 10 mg of freshly prepared plasma membranes) were assembled into infrared cells with BaF_2 windows and a path length of 50 μm. The infrared cell was mounted onto a thermostated sample holder and immediately placed in a Fourier-transform infrared spectrometer (Digilab FTS-11) equipped with a high-sensitivity mercury–cadmium telluride detector. Data acquisition was fully automated so that one spectrum was collected every 15 s (25 coadded interferograms) while the temperature was raised at 1 °C/min from about 20 to 45 °C in the case of the live cells and from about 15 to 50 °C in the case of the isolated plasma membranes. Interferograms were coadded with a mirror velocity of 1.26 cm s^{-1} and a maximum optical retardation of 0.25 cm, triangularly apodized, zero-filled twice, and Fourier transformed to yield 8 cm^{-1} resolution spectra with encoding every 2 cm^{-1}. Typically, 100 individual infrared spectra were collected during 25 min. For each of the three fatty acid's growth, the entire experiment was repeated at least 3 times, both for the live cells and for the isolated membranes. In all cases, consistent results were obtained. After each experiment with live *A. laidlawii* cells, the viability of the cells was determined as mentioned earlier. The frequencies and widths of infrared absorption bands were obtained with an uncertainty of less than 0.1 cm^{-1} using algorithms developed in our laboratory (Cameron et al., 1982; Cameron & Moffatt, 1984).

Results and Discussion

The infrared spectra obtained from live *Acholeplasma laidlawii* cells are extremely complex as they contain bands

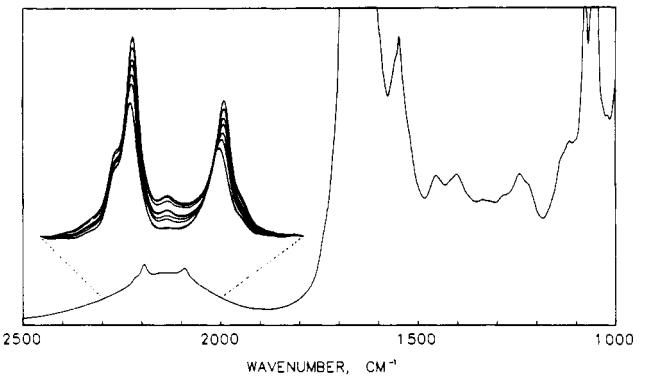

FIGURE 1: Infrared absorbance spectrum of live *Acholeplasma laidlawii* B cells obtained from a growth medium supplemented with hexadecanoic-d_{31} acid and avidin. The inset shows a blowup of the carbon–deuterium stretching bands (after water subtraction) at eight different temperatures between 20 and 45 °C.

from a variety of biomolecules (lipids, proteins, nucleic acids, and carbohydrates), as well as from water and inorganic ions, all of which are present in the live bacteria. However, in the case of *A. laidlawii* cells grown on a medium supplemented with perdeuterated fatty acids, the spectral region between 2000 and 2300 cm^{-1} does not contain absorption bands other than those originating from carbon–deuterium stretching vibrations. Since the deuterated fatty acids are incorporated biosynthetically only into the plasma membrane in the form of deuterated lipids, these bands can be used as a highly specific probe for the in vivo temperature response of the plasma membrane in *A. laidlawii* cells. The exact position (or "frequency") of the C–H (or C–D) stretching bands of lipid acyl chains provides a sensitive measure of the degree of conformational disorder of the membrane lipids [see Amey & Chapman (1983) and Casal & Mantsch (1984) and references cited therein].

Figure 1 shows the infrared spectrum between 2500 and 1000 cm^{-1} of live cells of *Acholeplasma laidlawii* B recorded at room temperature. The spectral region between 2300 and 2000 cm^{-1} contains the C–D stretching bands superimposed on a broad absorption band of water (Cameron et al., 1979). The inset shows these bands more clearly at several temperatures after subtraction of water. The strong bands at 2196 and 2090 cm^{-1} represent the CD_2 antisymmetric and CD_2 symmetric stretching bands, respectively, whereas the weaker bands at about 2212 and 2155 cm^{-1} are the CD_3 asymmetric and CD_3 symmetric stretching bands, respectively. There are two characteristics of these bands which render them useful in studies of lipids and biomembranes. First, the mechanism of interaction between the head group and the individual CD_2 groups, and hence the perturbing effect of the head moieties on the vibrational modes of the acyl chains, is inductive. Consequently, once the chain has reached a length of about six carbon atoms, the head group has effectively no direct electronic effect on the frequencies, bandwidths, and intensities of the CD_2 and CD_3 stretching bands. Second, the carbon–deuterium bonds are virtually immune to polar interactions such as hydrogen bonding, and, in fact, the only factors that significantly affect the shapes and frequencies of these infrared absorption bands are the degree of conformational disorder and the mobility of the acyl chains.

Changes are evident in both the peak maxima (frequency) and the shape (width) of all the C–D stretching bands in Figure 1. The infrared spectra of the membranes in the live cells at temperatures below 20 °C are characteristic of membranes in the gel phase (Casal & Mantsch, 1984). However, when the temperature of the sample with the live cells increases, the C–D stretching bands broaden, shift to higher frequencies, and decrease in intensity. The infrared spectra at around 40 °C are characteristic of membranes in the conformationally disordered liquid-crystalline phase. These changes in infrared spectral characteristics are identical with those observed at the gel to liquid-crystal phase transition in the spectra of deuterated model membranes (Sunder et al., 1978; Casal et al., 1980; Cameron et al., 1981; Dluhy et al., 1983; Lee et al., 1984; Mendelsohn et al., 1984a–c).

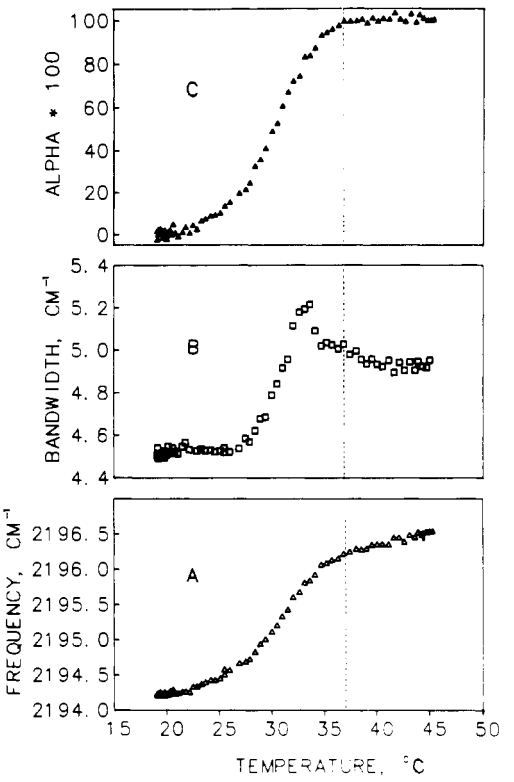

FIGURE 2: Temperature dependence of the frequency (A) and width (B) of the CD_2 antisymmetric stretching band of deuterium-labeled membranes in live *A. laidlawii* cells; displayed in panel C is the conformational index, α. Frequencies are determined as the center of gravity of the topmost 8–10 cm^{-1} segment of the band, while bandwidths are determined at 0.95 peak height. Each curve is derived from 80 individual spectra; the dotted tracer line indicates the temperature of growth.

Each of the two strong CD_2 stretching bands can be used conveniently to describe the conformational state of the acyl chains, although the symmetric mode undergoes larger changes with temperature. A low frequency is characteristic of highly ordered (predominantly trans) acyl chains, while higher frequencies reflect the progressive introduction of conformational disorder, i.e., gauche rotamers in the acyl chains. The increase in frequency is accompanied by an increase in bandwidth, which reflects the increased mobility of the acyl chains in the liquid-crystalline phase. By the use of appropriate algorithms, the frequency and width of these bands can be precisely measured as a function of temperature, leading to plots such as those shown in Figure 2. While a phase transition is well-defined by both parameters, it should be noted that the maximum rate of change of the bandwidth (Figure 2B) does not coincide with the maximum rate of change of the frequency (Figure 2A). Furthermore, the plot of width vs. temperature passes through a maximum at a temperature at which the frequency is still increasing. This is not observed in the spectra of systems that undergo first-order phase transitions; however, it has been observed in the spectra of other systems in which two or more phases coexist during a phase transition (Ume-

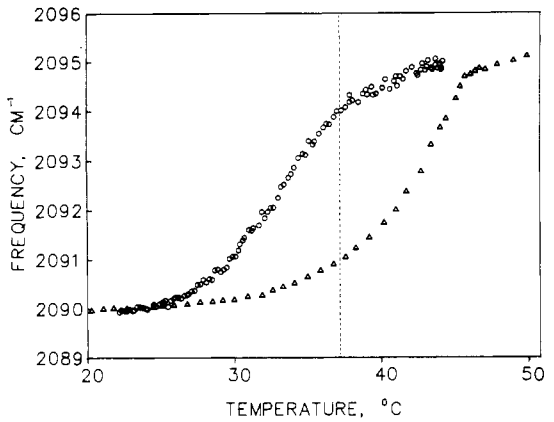

FIGURE 3: Comparison between the temperature dependence of the frequency of the CD_2 symmetric stretching band of membranes labeled with C16:0-d_{31} in live *A. laidlawii* cells (circles) and in isolated membranes (triangles). The temperature of growth, i.e., 37 °C, is marked by a dotted line.

mura et al., 1981; Dluhy et al., 1983). The variation in bandwidth then reflects the overlap of absorption bands due to different species.

The relationship between the changes in the frequency or width of a CD_2 stretching band and the degree of conformational disorder in the system is not linear. Thus, while the frequency or width plots can be used for comparisons between different systems, an attempt at quantitation of the proportions of the gel and liquid-crystalline phases requires the use of a model. The model we employ, which closely resembles that used in NMR studies (Jarrell et al., 1981), is described elsewhere (Dluhy et al., 1985). In brief, it assumes that any spectrum, S_T, observed at a temperature which falls within the range of the phase transition is given by

$$S_T = (1 - \alpha)S_T^{g} + \alpha S_T^{lc}$$

where the conformational index (α) is the fraction of the liquid-crystalline phase present at the temperature T ($0 < \alpha < 1$) and S_T^{g} and S_T^{lc} are, respectively, the gel and liquid-crystalline phase spectra expected at this temperature if there had been no transition. S_T^{g} and S_T^{lc} are computed by extrapolation of gel and liquid-crystalline phase spectra recorded at temperatures below the onset and above the completion of the transition. Figure 2C illustrates a plot of the percent liquid-crystalline phase vs. temperature. The advantage of this plot over the frequency plot is that the conformational index α varies linearly with the degree to which the transition has proceeded and provides a better means of comparing data obtained under different conditions.

Figure 3 compares the temperature profile of the CD_2 symmetric stretching frequency of membranes in live *A. laidlawii* cells grown at 37 °C on C16:0-d_{31} with that of the isolated membranes obtained from this particular growth. Both the labeled lipids in the membranes of live cells and the labeled lipids in the membranes isolated from the cells undergo a typical gel to liquid-crystal phase transition. In both cases, the membranes are in an ordered gel phase at temperatures below 25 °C and in a conformationally disordered, more "fluid" phase at temperatures above 45 °C. This is clearly demonstrated by the values of the corresponding C–D stretching frequencies, which, at temperatures below 25 °C and above 45 °C, are identical in membranes of live cells and in isolated membranes. However, the shapes of the temperature profiles, i.e., the frequencies at a given temperature between 25 and 45 °C, are drastically different. Thus, at 37 °C, i.e., the temperature of growth, the phase transition is

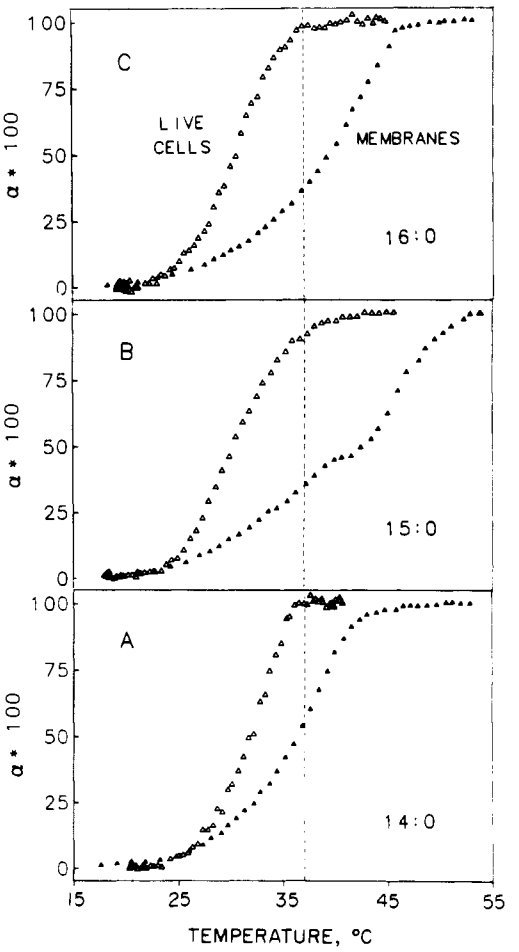

FIGURE 4: Fractional population of lipids in the liquid-crystalline phase in isolated membranes (filled triangles) and in membranes of live *Acholeplasma laidlawii* cells grown on C14:0-d_{27} (A), C15:0-d_{29} (B), and C16:0-d_{31} (C).

completed in the case of live cells, whereas it has hardly commenced in the case of isolated membranes.

In order to quantitate the fractional population of the liquid-crystalline phase at a given temperature in the range of the phase transition, we have applied the procedure outlined earlier to all the spectra of live cells as well as to those of the membranes obtained after cell lysis. Figure 4 illustrates plots of α, the fractional population of the liquid-crystalline phase, vs. temperature for membranes of *A. laidlawii* cells grown at 37 °C on C14:0-d_{27} (Figure 4A), C15:0-d_{29} (Figure 4B), and C16:0-d_{31} (Figure 4C). In each case, the α values obtained from the membranes in live cells are compared with the corresponding α values from the isolated membranes.

An examination of the data in Figure 4 reveals a number of differences between the thermal responses of isolated membranes and those of membranes of live cells. Foremost, the introduction of conformational disorder (i.e., the acyl chain melting process) occurs over a narrower temperature range in the membranes of live cells, indicating a higher degree of cooperativity compared to the transition in isolated membranes. Furthermore, the degree of conformational disorder found at a given temperature in the range of the phase transition is always higher in the membranes of live cells. As shown in Table I, the membranes of live cells grown at 37 °C are practically all fluid at the temperature of growth. Interestingly, we found no significant effect of chain length on the transition in the membranes of live *A. laidlawii* cells. The temperature response of live cells enriched in C14:0-d_{27} (83%), C15:0-d_{29} (99%), and C16:0-d_{31} (71%) is, for most purposes, identical.

This, however, is not the case for the isolated membranes, which show significant differences in their temperature profiles; that is, membranes containing lipids with shorter acyl chains are more fluid at a given temperature. Thus, at 37 °C, the fractional population of membrane lipids in the liquid-crystalline phase is 58% for isolated membranes enriched in C14:0-d_{27} and only 38% for isolated membranes enriched in C16:0-d_{31} (see Table I). Membranes grown on pentadecanoic acid show peculiar temperature profiles which cannot be easily compared with those of membranes enriched in C14:0 and C16:0 (Casal et al., 1982).

Since the membranes investigated in this study are highly enriched in a single saturated acyl chain with 14, 15, or 16 carbon atoms, a possible way the microorganism can modify its fluidity at a given temperature is to modify the nature of the lipid head group (Wieslander et al., 1980). We have found exactly this. To accommodate the variation in chain length, the *A. laidlawii* cells grown at 37 °C respond by producing different amounts of lipids with monoglucosyl diglyceride and diglucosyl diglyceride head groups. That is, an increase in chain length (at a given temperature of growth) results in a marked increase in the amount of monoglucosyl diglyceride relative to diglucosyl diglyceride. As shown in Table I the MGDG/DGDG ratio varies by a factor of 16 between membranes enriched in C14:0-d_{27} and those enriched in C16:0-d_{31}.

Concluding Remarks. The present investigation illustrates the usefulness of the infrared spectroscopic technique for studying the effect of temperature on the structure of membranes in live cells. Besides demonstrating that the membrane lipids in live cells undergo conformational changes reminiscent of the gel to liquid-crystal phase transition in lipid model membranes, our results provide evidence that the thermal response of membrane lipids in live cells differs from that of lipids in isolated membranes. These findings are important since it is usually inferred that results obtained from isolated membranes also apply to the membranes in live systems. Our data, on the other hand, do not provide an explanation as to how the living organism adjusts the cooperativity of its gel to liquid-crystal phase transition and how it controls the conformational changes that occur at temperatures around the temperature of growth. Naturally, this leaves room for speculations and suppositions. One possible cause of the differences in the fractional amount of liquid-crystalline phase present at a given temperature in isolated membranes and in membranes in live cells could be the lipid and protein asymmetry in native intact membranes and the existence of a potential in live cells which is absent in isolated membranes. Further experiments are currently under way in our laboratory, including an investigation into the effect of a potential created by a sodium–potassium gradient in model membranes.

Registry No. C14:0, 544-63-8; C15:0, 1002-84-2; C16:0, 57-10-3.

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