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Acholeplasma laidlawii Membranes: A Fourier Transform Infrared Study of the Influence of Protein on Lipid Organization and Dynamics†

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ABSTRACT: Fourier transform infrared spectroscopy is applied to the study of intact and deproteinated plasma membranes of *Acholeplasma laidlawii*, enriched biosynthetically with perdeuteriopalmitoyl chains. The temperature-dependent behavior is monitored via the CD stretching modes and compared with that observed in the model membrane 1,2-diperdeuteriopalmitoyl-*sn*-glycero-3-phosphocholine. The broad ramplike transition observed in the natural membranes is shown to consist of two overlapping stages. In the lower temperature range the principal change is a reduction in the rigidity of the lipid matrix. Subsequently, over a 5 °C range, centered at the growth temperature, a large change in the gauche/trans conformer ratio of the acyl chains occurs, similar to that generally observed in model systems. This is followed by an abrupt cessation of the phase transition. The effects of membrane protein on the phase transition are shown to be relatively minor. Firstly, they produce a decrease in the rate of acyl chain motion at a given temperature, resulting in a reduction in the width of the transition. In addition, the presence of protein increases the population of gauche conformers of the fatty acyl chains in the liquid-crystalline phase of the membrane lipids.

The nature and the state of lipids are believed to regulate not only the architecture and physical properties of biological membranes but also various physiological functions that ensure the viability of the organism; for example, enzyme activities depend to a large extent on the "fluidity" of the lipid matrix (Baldassare et al., 1977; Racker et al., 1975), which, in turn, is a function of temperature and of the lipid composition.

A number of such functional requirements have been ascribed to specific lipid-protein interactions or to the presence of lipoprotein subunits which are believed to form channels for the transport of metabolites or to stabilize the active conformation of lipid-bound enzymes. Such lipid-protein interactions could influence the behavior of the endogenous lipid pool, such as thermotropic mesomorphism, the cooperative change from a solidlike gel state to a fluidlike liquid-crystalline state.

We have focused our attention on *Acholeplasma laidlawii*, a microorganism capable of regulating the "fluidity" of its plasma membrane when the growth temperature or the external fatty acid source is varied (McElhaney, 1974). The enzymatic activity in *A. laidlawii* has been found to depend critically on the phase behavior of the membrane lipid pool, as recently determined with the membrane-bound ATPase (Silvius et al., 1980).

The phase transition of *A. laidlawii* plasma membrane lipids has been studied by different physical techniques such as calorimetry (Steim et al., 1969), X-ray diffraction (Engelman, 1970), NMR[1] (Smith et al., 1979), ESR (Huang et al., 1974; Butler et al., 1978), and IR (Casal et al., 1979). In all cases it was found that the onset of molecular disorder in the lipid fatty acyl chains occurs over a wide temperature range. This is in contrast to model membrane systems where the phase transition is very sharp and complete in less than 1 °C (Sunder et al., 1978). The broad temperature range of the phase transition in *A. laidlawii* membranes could be explained either by lipid-protein interactions which modulate the thermal behavior of the lipid matrix or by heterogeneity of its lipid pool (McElhaney, 1974). Recent studies with *A. laidlawii* membranes enriched to >95% in a particular fatty acid have revealed much narrower phase-transition ranges, supporting the latter explanation (Silvius & McElhaney, 1978).

In order to obtain information regarding the influence of proteins on the degree of organization and the dynamics of the lipid components in a natural membrane, we have performed a detailed Fourier transform infrared study of the plasma membrane of *A. laidlawii* grown on perdeuteriopalmitic acid and of the lipid matrix extracted from the same membrane. This microorganism readily incorporates deuterium-labeled fatty acids into its membrane lipids (Stockton et al., 1975, 1977), providing a direct probe of the membrane structure via the infrared C-D stretching bands. We are able to monitor small variations in these bands and recently demonstrated this ability in a report on the phase transition of *A. laidlawii* grown on perdeuteriopalmitic acid (Casal et al., 1979). In this publication we present a more detailed study of this phase transition and compare it with that observed in the lipids remaining after the removal of the proteins from the membranes and with the behavior of the model membrane DPPC-d_{62}. Considerable insight is obtained into the various types of motion performed by the fatty acyl chains due to the

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[1] Abbreviations used: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, 1,2-diperdeuteriopalmitoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance; ESR, electron spin resonance; FT-IR, Fourier transform infrared; DSC, differential scanning calorimetry.

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different responses of the several IR spectral parameters to motional effects. Particular emphasis is placed on the study of the frequency shifts and half-bandwidths of the symmetric CD_2 stretching mode. These can be related to specific phenomena: the frequency shifts to the melting of the chains or the introduction of gauche conformers and the half-bandwidths to variations in the rates and amplitudes of the translational and rotational motions of the CD_2 groups and the acyl chains. It is important to distinguish between the degree of organization of the fatty acid chains (gauche/trans conformer ratio) and the rate of motion within the ordered environment (Smith, 1979). Both contribute to what has been described as the "fluidity" of the lipids.

Materials and Methods

Chemicals and Biochemical Procedures. A. laidlawii B was grown at 37 °C in a medium supplemented with palmitic-d_{31} acid; plasma membranes were prepared as described earlier (Stockton et al., 1975). The composition of the fatty acyl chains, determined by gas–liquid chromatography and expressed in mole percent, was the following: 12:0, 7.0 mol %; 14:0, 26.6 mol %; 14:1, 2.0 mol %; 16:0, 64.5 mol %. For lipid analysis, the membranes were hydrolyzed and methylated in 0.7 N methanolic HCl and extracted with petroleum ether (bp 30–60 °C). The methyl esters of the fatty acids were analyzed on a Hewlett-Packard HP5710A gas chromatograph with 15% stabilized DEBS on Chromosorb W, A/W, 80–100 mesh, columns at 175 °C. Heptadecanoic acid was used as the internal standard.

The membrane lipids were extracted as follows. Twenty-five milligrams of membrane was taken up in 2 mL of H_2O, and then 5 mL of CH_3OH and 2.5 mL of $CHCl_3$ were added and the mixture was stirred for 4 h at room temperature. After centrifugation, the precipitate was reextracted with CH_3OH–$CHCl_3$ (2:1 v/v). The $CHCl_3$ phase was dried over Na_2SO_4, filtered, and dried under nitrogen; 3.4 mg of lipids was obtained.

1,2-Diperdeuteriopalmitoyl-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) was obtained from Lipid Specialties Inc., Boston. Its purity was confirmed by a single spot on thin-layer chromatography; polarimetry measurements indicated that the natural *sn*-3 stereochemistry was retained.

Sample Preparation. Lyophilized *A. laidlawii* membranes (5 mg) were hydrated with 1 mL of doubly distilled water and centrifuged at 8000*g*, and the supernatant was removed. The fully hydrated membranes were then placed in the center of a CaF_2 window assembled into a temperature-controlled 50-μm thick infrared cell. The lipids extracted from the *A. laidlawii* plasma membrane (3 mg) were deposited on a CaF_2 window and hydrated with doubly distilled water, and the window was assembled into a 25-μm temperature-controlled cell. Multibilayer dispersions of DPPC-d_{62} were prepared as described earlier and assembled into a 12-μm CaF_2 cell (Cameron et al., 1979).

Spectra. FT-IR spectra were recorded between 0 and 55 °C by using a Nicolet 7199 Fourier transform infrared spectrometer equipped with a deuterated triglycine sulfate detector and purged with nitrogen. Interferograms were collected with an optical retardation of 1 cm, apodized with a Happ-Genzel function, and transformed to yield a resolution of 0.9 cm^{-1}. The cell was placed in a thermostated mount with three degrees of freedom; an alignment laser was used to center the beam on the sample. Temperature was monitored by a copper–constantan thermocouple located against the edge of the cell window; the thermocouple output was routed to a printer via a Newport digital pyrometer. The spectrometer controls the entire process of recording a spectrum, triggering a print of the temperature, incrementing the temperature, waiting for temperature equilibration, and recording another spectrum.

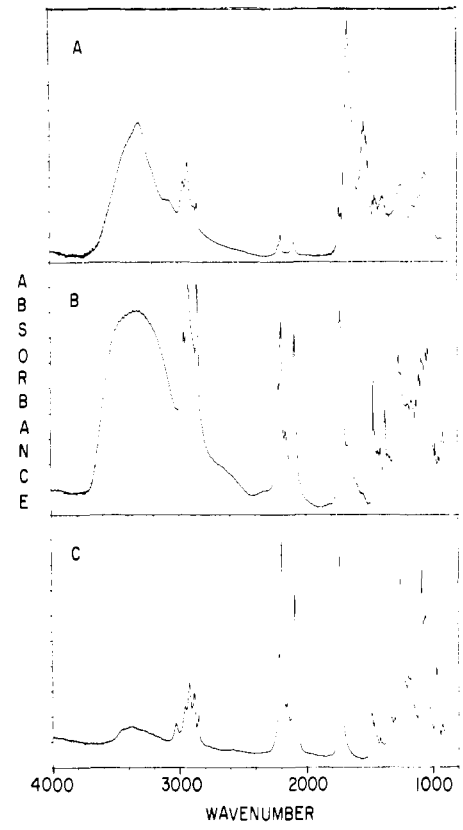

FIGURE 1: Fourier transform IR absorbance spectra at 25 °C of unhydrated samples of (A) intact *A. laidlawii* plasma membranes (grown at 37 °C on a medium supplemented with palmitic-d_{31} acid) recorded as a pellet composed of 1 mg of lyophilized membranes and 100 mg of KBr, (B) lipids extracted from the *A. laidlawii* membranes in (A) recorded as a thin film deposited on a CaF_2 window, and (C) DPPC-d_{62} recorded as a thin film deposited on a CaF_2 window.

Spectra were processed by using the techniques described in detail in a previous publication (Cameron et al., 1979). Difference spectra were generated by ratioing the spectra recorded at two different temperatures; the resultant spectrum comprises all changes occurring in that temperature interval. From such spectra, a parameter ΔA, the maximum change in absorbance in the region of a given peak, can be measured and normalized with respect to temperature to produce the parameter $\Delta A/°C$. Absorbance spectra were obtained by ratioing single-beam spectra against an empty background. Half-bandwidths and frequencies were measured from absorbance spectra after subtracting the water background. The water spectra used in these subtractions were measured at the same temperatures as the sample spectra in a cell of the same path length with the same type of window. Frequencies were also checked against those obtained directly from the single-beam spectra.

Results

Spectral Characteristics. As shown in Figure 1, the deuterium-labeled fatty acyl chains in the plasma membrane of *A. laidlawii* and in DPPC lead to characteristic signals in the infrared spectra. The acyl chain CD_2 stretching vibrations are located in the relatively unobstructed 2400–2000-cm^{-1} window and can be used to probe the lipid organization.

Figure 1A shows the infrared spectrum of lyophilized *A. laidlawii* membranes. The carbon–deuterium stretching modes

are evident as weak bands in the 2400–2000-cm^{-1} region in a spectrum dominated by protein absorptions. Strong amide A and B bands are evident at 3300 and 3100 cm^{-1}. Amide I and II bands appear at 1650 and 1530 cm^{-1}, respectively; their frequencies and relative intensities indicate that the dominant conformation adopted by the *A. laidlawii* membrane proteins is α helical (amide I, 1650 cm^{-1}) with some random coil structure (amide I, 1535 cm^{-1}) (Thomas, 1977; Oldfield et al., 1972). There is no evidence of β structure. A strong C–H stretching band characteristic of proteins can be observed at 2978 cm^{-1} (Parker, 1971).

The spectrum of the lipid component of the membrane is shown in Figure 1B. This spectrum contrasts dramatically with that in Figure 1A. All the above-mentioned protein bands have disappeared, and the CD_2 bands are now the strongest in the spectrum; the characteristic antisymmetric and symmetric CD_2 stretching modes are observed at 2194 and 2089 cm^{-1}, respectively, with the terminal CD_3 modes evident at 2212 and 2169 cm^{-1}. The two CH_2 stretching modes at 2920 and 2856 cm^{-1} and the CH_2 scissoring mode at 1468 cm^{-1}, resulting from the nondeuterated lipid component (~20% by weight; Stockton et al., 1975) are characteristic of highly ordered acyl chains (Krimm et al., 1956; Koyama et al., 1977). The carbonyl band (1736 cm^{-1}) of the ester linkage in the head groups is now very strong, whereas previously it was only evident as a shoulder on the side of the amide I band. In the fingerprint region there are many sharp bands between 1400 and 1000 cm^{-1}, typical of solid-state carbohydrate spectra (Vasko et al., 1972). These probably result from the high proportion of mono- and diglucosyl lipids (~50% of the lipid component) found in the membranes of *A. laidlawii* (Wieslander & Rilfors, 1977). The OH stretching band at 3400 cm^{-1} can also be associated with the carbohydrate component, although it is partly due to residual water, as indicated by the weak H_2O bending mode at 1640 cm^{-1}.

Figure 1C shows the spectrum of a deuterated phospholipid, DPPC-d_{62}. The CD_2 stretching modes and the carbonyl band are almost identical with those of the *A. laidlawii* lipids; the C–H stretching bands between 3100 and 2800 cm^{-1} are due to the CH bonds of the glycerol and choline components, as well as to the CHD stretching modes of incompletely deuterated acyl chains. The fingerprint region is much simpler than that of Figure 1B, the dominant bands being the phosphate modes at 1230 and 1090 cm^{-1} and the ester COC stretching bands at 1170 and 1070 cm^{-1}.

The spectra in Figure 1 illustrate the general features of the FT-IR spectra of intact and deproteinated *A. laidlawii* plasma membranes, compared to those of a single phospholipid. However, in order to monitor their biologically relevant properties, we studied these membrane preparations in the presence of a large excess of water. The FT-IR spectra of the C–D stretching region of such samples are shown in Figure 2. While water is an intense infrared absorber, the C–D stretching modes occur in a region of relatively low water absorption. There is, however, a "water association" band in this region, and although this is the weakest band in the mid-IR spectrum of water, it can dominate the spectrum of a biological sample if a large quantity of water is required to ensure complete hydration. This is the case in Figure 2A, the spectrum of fully hydrated *A. laidlawii* membranes. The C–D stretching modes are only evident as weak features on top of the broad water band, while most other regions of the spectrum (not shown in this figure) are opaque to infrared radiation, the energy having been absorbed by the various water bands. The ratio of water to sample peaks is determined by the affinity

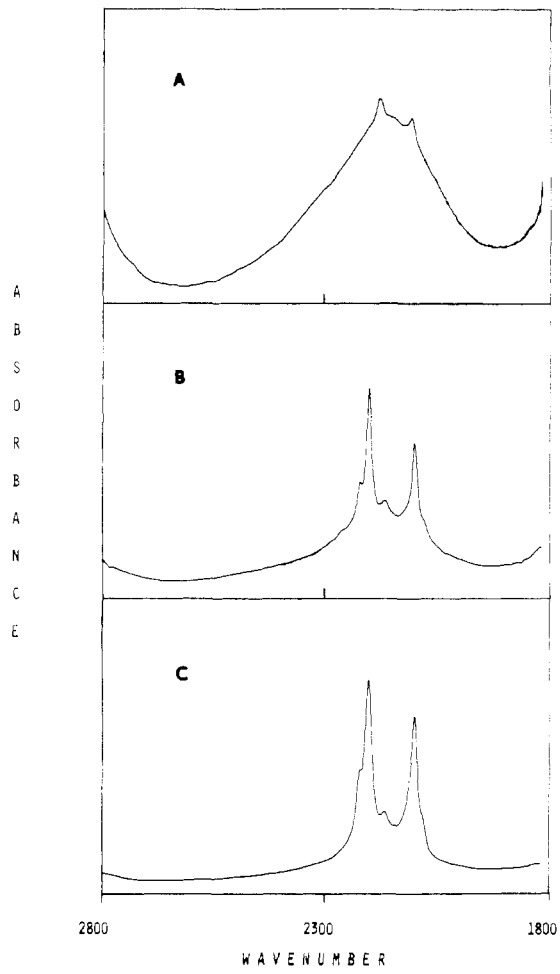

FIGURE 2: FT-IR absorbance spectra at 25 °C of the CD stretching region of fully hydrated samples of (A) intact *A. laidlawii* plasma membranes (50-μm cell), (B) deproteinated plasma membranes (25-μm cell), and (C) DPPC-d_{62} model membranes (12-μm cell).

of the sample for water, and consequently the spectrum cannot be improved by addition of more sample or further centrifugation. As shown in Figure 2B, the removal of proteins, and the consequent change in the proportion of lipids in the sample, produces an IR spectrum of the fully hydrated deproteinated membrane with considerably less interference from the water absorption. In the case of DPPC-d_{62} multibilayer dispersions (Figure 2C), the water band is even weaker and is only evident as a slight background curvature. In all cases hydration results in a general broadening of the IR bands, as expected for the increased mobility of the lipids in the hydrated samples (Hsia et al., 1970; Levine & Wilkins, 1971).

Absorbance Changes. Despite the water background, a technique outlined in detail elsewhere (Cameron et al., 1979) permits us to monitor quantitatively several IR spectral parameters as a function of temperature. The most readily obtained is $\Delta A/°C$, the maximum rate of change of absorption with temperature in the region of a given band. As it monitors simultaneously variations in peak height, peak position, and bandwidth, it is an excellent monitor of phase changes and provides a useful basis for comparing individual systems. Figure 3 shows the antisymmetric and symmetric CD_2 and CD_3 stretching modes of the deuterated acyl chains in deproteinated *A. laidlawii* membranes at two different temperatures. All bands show variations in position, height, width, and integrated intensity. The upper trace in this figure shows the corresponding FT-IR difference spectrum from which the ΔA parameter is measured.

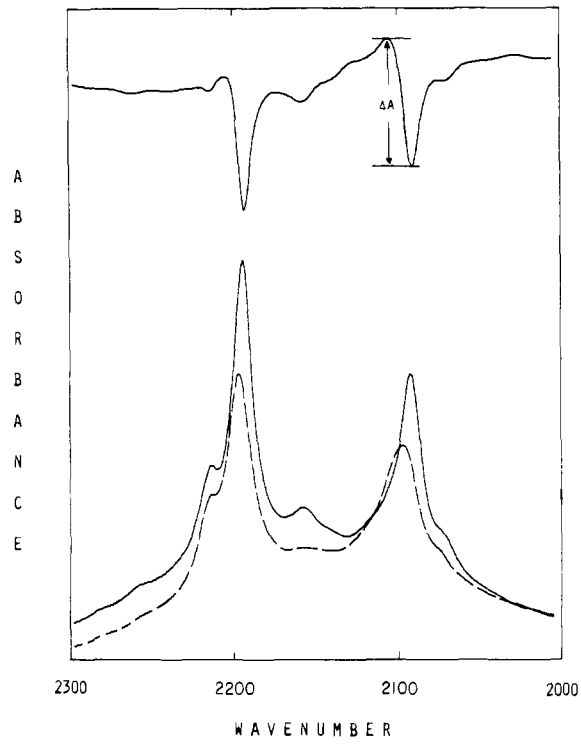

FIGURE 3: (Bottom) FT-IR absorbance spectra of deproteinated plasma membranes of *A. laidlawii* (grown on a medium supplemented with palmitic-d_{31} acid), in the CD stretching region at 15 °C (solid line) and at 42 °C (dashed line). (Top) Infrared difference spectrum obtained by subtracting the 15 °C spectrum from that obtained under otherwise identical conditions at 42 °C.

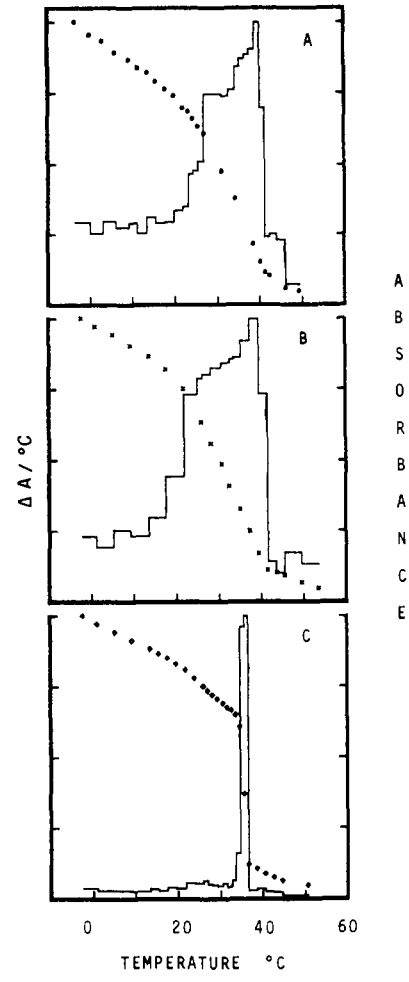

FIGURE 4: Temperature dependence of the symmetric CD_2 stretching vibration of the perdeuteriopalmitoyl chains in (A) the intact plasma membrane, (B) the deproteinated plasma membrane, and (C) the DPPC-d_{62} model membrane, as absorbance vs. temperature (dotted lines) and as the rate of change per degree vs. temperature (solid lines). On the ordinate axis all plots have been normalized between 0 and 1; consequently, quantitative comparisons cannot be made.

The results of such measurements are displayed in Figures 4A–C for all three systems, along with absorbance vs. temperature plots, readily derived from the $\Delta A/°C$ values. The thermal response of the model membrane system in Figure 4C is typical of a pure phospholipid. A maximum rate of change for DPPC-d_{62} is observed in a narrow temperature range centered at 37.5 °C (Sunder et al., 1978) with minor perturbations evident before and after the gel to liquid-crystal phase transition. This sharp response reflects the melting of the acyl chains of DPPC-d_{62} and the consequent drastic changes in all IR spectral parameters.

In the cases of the intact (Figure 4A) and deproteinated (Figure 4B) *A. laidlawii* plasma membranes, changes are observed over a much wider temperature range. The $\Delta A/°C$ plots show that, although it is much broader in the biological membrane, the transition is highly structured in both cases. The transition has the form of a ramp, as observed previously by DSC (Steim et al., 1969), FT-IR (Casal et al., 1979), and ^2H NMR (Davis et al., 1980). However, although intact and deproteinated membranes were previously studied by DSC (Chapman & Urbina, 1971), it was not evident from such studies that there are subtle differences between the two systems. This is apparent in the infrared data. Parts A and B of Figure 4 are identical above and in the region of the growth temperature. Below this temperature, in the case of the intact membrane, the rate of change decreases steadily and achieves a constant value at ~22 °C. The plot obtained from the deproteinated membrane is more plateaulike. The total width of the phase transition in deproteinated membranes is also greater, the onset occurring at about 15–18 °C compared to 22 °C in the case of the intact membrane.

Frequency Shifts. More commonly employed IR parameters are the frequency ($\bar{\nu}$) and the half-bandwidths ($\Delta\bar{\nu}_{1/2}$) of the individual vibrational modes. These parameters have the advantage of relating to specific phenomena at the molecular level and can be used to discriminate between different motions of the absorbing groups.

The frequency of a vibrational absorption is determined by the nature of both the vibrational mode and the vibrating group. The C–H and C–D stretching vibrations in acyl chains are decoupled from other vibrations occurring within the molecule; the bulk of the acyl groups are electronically decoupled from the polar head groups. Consequently, in the all-trans conformational state, each of these absorptions is observed at its characteristic frequency, regardless of the terminal functional groups, and is shifted only slightly as a result of interactions with molecules of a different class (e.g., proteins). However, the effects of changes in conformation, specifically the introduction of gauche conformers, are large, producing shifts to higher frequencies. Consequently, one can use these shifts to determine the introduction of gauche conformers or chain melting, the magnitudes of the shifts being related to the number of gauche bonds.

The largest variation in frequency in the acyl C–D stretching vibrations occurs in the symmetric CD_2 mode at 2090 cm^{-1}, with all effects being reflected to a lesser extent in the asymmetric mode (Sunder et al., 1978). The temperature dependence of the frequency of the symmetric vibration is shown in Figure 5 for all three systems. In the temperature range

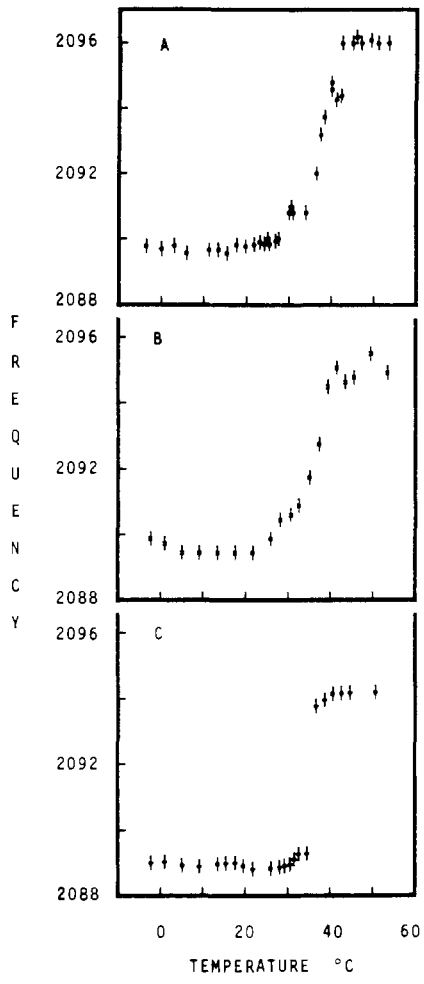

FIGURE 5: Temperature dependence of the frequency (in cm^{-1}) of the maximum of the CD_2 symmetric stretching vibration of the perdeuteriopalmitoyl chains in (A) intact plasma membranes, (B) deproteinated plasma membranes, and (C) DPPC-d_{62} model membranes.

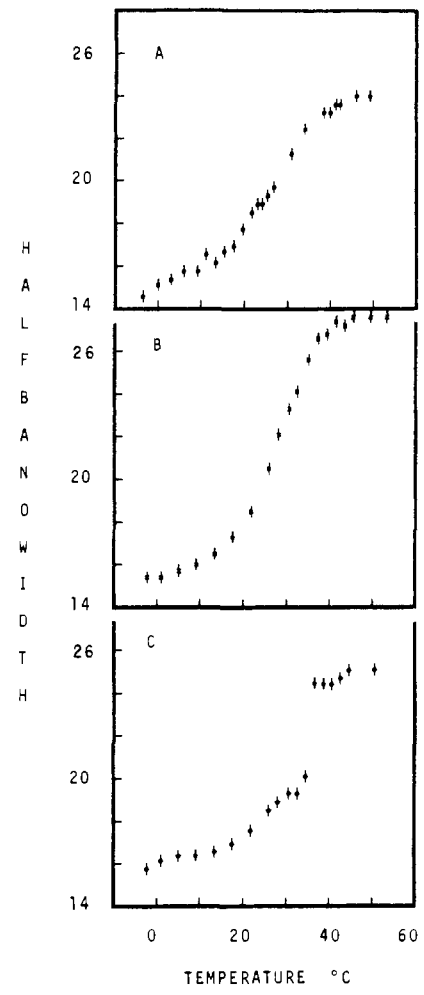

FIGURE 6: Temperature dependence of the half-bandwidths (in cm^{-1}) of the symmetric CD_2 stretching vibration of the perdeuteriopalmitoyl chains in (A) intact plasma membranes, (B) deproteinated plasma membranes, and (C) DPPC-d_{62} model membranes.

0–15 °C, which we shall refer to as that of the gel phase, the frequencies of both intact and deproteinated membranes are ~0.5–0.75 cm^{-1} higher than that observed in the model membrane. Above 15 °C we observe changes in frequency in all three situations. In the case of the DPPC-d_{62} model membrane (Figure 5C), there is a large increase in the CD_2 frequency at the main transition (37.5 °C).

The total temperature range over which the frequencies are observed to change is 28–41 °C in the case of the intact membranes and 22–41 °C for the deproteinated membranes. However, the rate of change is not constant but increases with temperature, such that 80% of the total change in the case of intact membranes and 70% with deproteinated membranes occur within the range 35–40 °C.

The frequencies of the CD_2 bands in the liquid-crystalline phase reflect the average number of gauche conformers introduced into the system by the phase change. A large variation is observed among the three systems; intact membranes have the highest frequency, the deproteinated membranes are 1 cm^{-1} lower, and the model membrane is 1 cm^{-1} lower again.

Half-Bandwidth Variations. In principle, a considerable amount of information regarding molecular motion can be obtained from the absorption band contour by means of moment analysis and the study of correlation functions (Bailey, 1974). This is difficult in the case of systems such as hydrated membranes because the spectrum of the deuterated species must be isolated from the curved background of the water absorption. However, the half-bandwidth is much less susceptible than the above methods to the introduction of errors due to imperfect background correction. The width of the band contour results from rotational, translational, and/or collisional effects. Thus, the half-bandwidth monitors the freedom of motion of the absorbing group, i.e., the amplitudes and rates of motion within its immediate environment. The half-bandwidth will therefore reflect the main thermal transition where a considerable change in this environment occurs. It is also sensitive to other changes which do not introduce gauche conformers, such as a decreased freedom of librational or torsional motion of the chains.

The plots showing the half-bandwidths of the symmetric CD_2 stretching modes as a function of temperature are shown in Figures 6A–C for the three cases investigated. In the gel phase the half-bandwidths of the intact and deproteinated membranes are identical from 0 to 15 °C, increasing slowly with increasing temperature. In the model system we find a slightly higher half-bandwidth at 0 °C, but this value remains constant up to 15 °C, and all three systems show the same half-bandwidth between 10 and 15 °C. Above 15 °C the model system shows a steady increase followed by a large change at the main transition. Above this temperature the half-bandwidth remains constant.

The behavior observed in the two membrane systems is quite different from that of the model system. In the temperature range 20–40 °C, that is, the phase transition as indicated by

the $\Delta A/°C$ plot, the rates of change in half-bandwidth are greater than those observed in the gel phase (below 20 °C) and in the liquid-crystal phase (above 40 °C). In contrast to the variable rates of change observed in the $\Delta A/°C$ and in the frequency plots of the two membrane preparations, the rates of change in half-bandwidth over this range are relatively constant, indicating a continuous variation in freedom of motion.

Although the general behavior is the same in the intact and deproteinated lipids, a close examination shows that there are distinct differences. At 15 °C the half-bandwidths are identical but, as the temperature is increased, the rate of increase of half-bandwidth is greater in the deproteinated membranes. This results in a difference in half-bandwidth of 2 cm^{-1} at 30 °C and 4 cm^{-1} in the liquid-crystalline phase.

In light of the observation that the greater part of the frequency shift occurs in the temperature range 35–40 °C in the *A. laidlawii* membranes, we have examined carefully the half-bandwidth changes in the various temperature ranges. There is no evidence of a sharp change in the range 35–40 °C. In fact, in both systems there is a greater change in the range 20–30 °C than in the range 30–40 °C. Clearly, the phenomenon occurring between 20 and 40 °C is not just a change in conformational equilibrium of the acyl chains.

Discussion

The infrared parameters presented in this study are of three types. The $\Delta A/°C$ parameter is a general monitor of spectral changes and is sensitive to any effect producing changes in the IR spectrum. The CD_2 stretching frequencies, on the other hand, are sensitive to the introduction of gauche conformers (melting of the acyl chains) but, within these systems, are virtually insensitive to other effects (Jones & Sandorfy, 1956). The half-bandwidths fall into an intermediate category. They vary according to the freedom and rate of motion of the vibrating group; that is, the greater the amplitudes and rates of motion of the chain, the larger the half-bandwidth. Consequently, the half-bandwidth is also sensitive to the introduction of gauche conformers. In addition, variations in half-bandwidth can be related to other motions of the chains, such as libration of, or torsional motion about, the long axis and changes in intermolecular interactions. In all these latter cases, the frequency of the vibration remains constant.

Effect of Temperature. Comparing the IR data for the intact and deproteinated *A. laidlawii* membranes with those for DPPC-d_{62}, we find that below 15 °C the half-bandwidths of all three systems agree within experimental error. The frequencies of the intact and deproteinated membranes are almost identical and slightly higher than those of the model membrane. While small differences in frequency might be ascribed to the presence of some gauche conformers of the fatty acyl chains in the *A. laidlawii* membranes or to altered intermolecular interactions in the intact membrane, the close agreement between both parameters for all three systems indicates that the chain conformations, and the motions of the chains, are very similar.

The gel phases of DPPC-d_{62} and DPPC model membranes have been characterized in recent Raman (Gaber et al., 1978; Yellin & Levin, 1977) and ^2H NMR (Davis, 1979) studies. The all-trans conformation of the chain predominates. At 15 °C they undergo considerable motion about the long axis of the acyl chains, while at 0 °C the rate of this motion is greatly reduced. It is clear that in both the intact and deproteinated membranes the lipids are also in the all-trans conformation in the gel phase. The reduction in half-bandwidth with temperatures approaching 0 °C results from reduction of the amplitude of torsional motions. With regard to the intact membranes, the same conclusion was reached in a recent ^2H NMR study (Smith et al., 1979) where it was found that the band profiles of intact *A. laidlawii* membranes at 0 and 22 °C closely resembled those of the model membrane DPPC-d_{62}.

This similarity of the model and natural membranes in the gel state is not reflected in the nature either of the phase transition or of the liquid-crystalline phase. There is a clear distinction between the behavior of DPPC-d_{62} and the *A. laidlawii* systems in terms of the width of the transition and the values of the IR spectral parameters in the liquid-crystal phase.

Although dissimilar to that of the model system, the transitions of the intact and deproteinated *A. laidlawii* membranes are similar to one another, regardless of which IR parameter is compared. The $\Delta A/°C$ plots resemble those obtained by DSC and ^2H NMR; they are ramplike in form, cover a 20–25 °C temperature range, and terminate abruptly a few degrees above the growth temperature.

This general form of the phase transition has previously been suggested to result from the various proportions of different chain lengths in the lipids of the natural membranes. The phase transition is proposed to be a cooperative melting of the acyl chains, as in DPPC, with the shorter chains melting at lower temperatures and facilitating the melting of the longer acyl chains as the temperature is raised (Phillips et al., 1970). If this were the case, we would expect to observe in the infrared spectrum a simultaneous shift of the frequency of the band due to the introduction of gauche conformers and a broadening of the band as a consequence of the increased freedom of motion. However, a quite different behavior is observed. Almost all the frequency shifts observed in the biomembrane systems occur in the range 35–40 °C, demonstrating that the melting phenomenon is concentrated in a small range centered at the growth temperature (37 °C). Similar large fluctuations in this temperature range have been observed in the moments of ^2H NMR spectra of intact *A. laidlawii* membranes and have been related to a decrease in the order of the acyl chains (Smith et al., 1979; Davis et al., 1980).

In contrast to the behavior of the peak positions, the half-bandwidths change throughout the entire range from 20 to 40 °C. In fact, they undergo larger changes in the lower half of this range than in the upper half; i.e., changes in half-bandwidth are maximal in a range where only minimal variations in peak positions are found. Thus, in the 20–35 °C range the principal phenomenon is an increase in the freedom and rate of motion of the chains without a concomitant large increase in the number of gauche conformers. In the range 35–40 °C, the change is primarily associated with the melting phenomenon.

The increase in motional freedom in the range 20–35 °C must result from a more rapid and greater amplitude of reorientation about the acyl chain long axis and a decrease in the packing of the acyl chains, i.e., the restrictions imposed by forming a regular close-packed matrix. Ultimately, this loosening of the lipid matrix leads to a condition where gauche conformers are introduced, as evidenced by the frequency shifts commencing at ~28 °C in the intact membranes and at ~25 °C in the deproteinated membranes. In the 35–40 °C range, a change in the conformer population is the main phenomenon, and the broadening results from the introduction of gauche conformers. This interpretation is in complete accord with the conclusions of ^2H NMR studies in *A. laidlawii* membranes enriched in palmitic-*13,13*-d_2 acid (Smith et al., 1979).

Effect of Proteins. The above discussion on the effect of temperature was centered on the general form of the phase transition which is the same in the intact and deproteinated membranes. Clearly, the presence of proteins produces no dramatic changes in the nature of this transition. However, there are subtle differences between the two systems which do reflect the effects of the proteins.

Firstly, the introduction of proteins delays the onset of the phase transition by ~5–7 °C. This is particularly clear in the $\Delta A/°C$ plots, but is also evident from the frequency and half-bandwidth data. Although there is a delay in the onset of the transition, there is no effect on the temperature at which the transition terminates.

Secondly, the frequency shift in the temperature range 35–40 °C is greater in the case of intact membranes than for the isolated lipids, demonstrating that the presence of proteins results in a greater disordering of the fatty acyl chains (as measured by the increase in the number of gauche bonds) in the liquid-crystalline state of the intact membranes. Preliminary results from a recent ^2H NMR comparison of intact *A. laidlawii* membranes with their isolated lipids are in agreement with these IR data (R. Deslauriers, K. W. Butler, and I. C. P. Smith, unpublished experiments).

Thirdly, as shown by the fact that at all temperatures above 15 °C the half-bandwidths are greater in the IR spectra of the deproteinated membranes, the proteins limit the total freedom of motion of the lipid acyl chains in the gel phase during the transition and in the liquid-crystal phase. This occurs despite the fact that more gauche conformers are introduced into the intact membrane by the phase change (vide supra). The immobilization of membrane lipid by protein has been demonstrated in a variety of systems by other spectroscopic techniques (Marsh et al., 1978; Seelig & Seelig, 1978; Oldfield et al., 1978). These conclusions emphasize the inadequacy of the popular description of membrane lipid properties in terms of "fluidity". Despite a disordering of the acyl chains, their rates of motion are apparently slower in the presence of protein.

Conclusions

The results of this study on *A. laidlawii* membranes lead to conclusions with regard to two aspects, the detailed nature of the temperature-dependent behavior and the effects of protein on lipid conformation and dynamics.

The data demonstrate that the temperature-dependent behavior has the same general form in the intact and deproteinated membranes. The gel phase is a highly ordered rigid state, very similar to that found in the model system DPPC-d_{62}. The acyl chains are in the all-trans conformation undergoing limited librational and torsional motions. The transition occurs in two overlapping stages. At the onset of the transition, the principal effect is a decrease in the rigidity of the system, with no change in the gauche/trans conformer ratio of the acyl chains. In the intermediate stage, this effect continues but is accompanied by the gradual introduction of gauche conformers. This results in a condition of the membrane where a slight temperature perturbation produces a large change in the degree of conformational order and rate of motion of the fatty acyl chains and switches it into the liquid-crystal phase. As this effect is centered at the growth temperature, it is clearly a regulatory mechanism and is much more important than implied by the broad transitions observed by DSC and from the $\Delta A/°C$ parameter.

The effect of the endogenous proteins is to immobilize the lipid acyl chains in their preferred conformations at a given temperature. Thus, in the upper range of the gel phase and up to 35 °C, the intact membranes are more gellike than the deproteinated membrane lipids; the bands have smaller widths and lower frequencies. At the critical melting stage there is a greater change in frequency in the intact membranes; that is, the presence of proteins increases the number of gauche conformers present in the liquid-crystalline state. As the half-bandwidths are still narrower in the intact than in the deproteinated membranes, the proteins stabilize the gauche conformers in the liquid-crystal phase and thus decrease the rate of acyl chain interconversion between gauche and trans conformers.

The study of biomembranes by FT-IR provides detailed information concerning the physical state of lipids under conditions of varying composition and temperature. Further studies on deuterium-enriched biological systems will facilitate the understanding of the functions of the various membrane components and their interactions with one another.

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Quantitative Study of the Fluidity of *Escherichia coli* Membranes Using Deuterium Magnetic Resonance†

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ABSTRACT: Specifically deuterated palmitic acid was incorporated into the membrane phospholipids of the L51 strain of *Escherichia coli*. The cytoplasmic and outer membranes were separated by using standard techniques and studied by deuterium nuclear magnetic resonance between 0 and 40 °C. Distinctive liquid-crystalline and gel spectra were observed to coexist over a wide temperature range. The relative intensities of these spectra provided a direct measure of the fraction of the deuterium-labeled phospholipids in the fluid state as a function of temperature. Above 37 °C, the amount of immobilized or gel-phase phospholipid is estimated to be less than 3% of the total phospholipid. The gel to liquid-crystalline transition region for the outer membrane was shifted upwards by ~7 °C relative to that of the cytoplasmic membrane, in agreement with previous studies [Davis, J. H., Nichol, C. P., Weeks, G., & Bloom, M. (1979) *Biochemistry 18*, 2103]. The orientational order in the fluid phase of both membranes decreased gradually with increasing temperature and was greater in the outer membrane than in the cytoplasmic membrane. The orientational order of the gel-phase component was the same for both membranes, within an experimental uncertainty of 10%, and was independent of temperature from 0 to 30 °C for the outer membrane and from 10 to 30 °C for the cytoplasmic membrane.

The membrane phospholipids of *Escherichia coli* undergo a broad gel to liquid-crystalline phase transition which has been studied by a variety of techniques, including differential scanning calorimetry (Baldassare et al., 1976; Jackson & Sturtevant, 1977; Jackson & Cronan, 1978), X-ray diffraction (Overath et al., 1975; Schechter et al., 1974; Linden et al., 1977; Harder & Banaszak, 1979), electron spin resonance (Rottem & Leive, 1977; Linden et al., 1973; Sackmann et al., 1973), and fluorescence (Overath & Träuble, 1973; Cheng et al., 1974; Overath et al., 1975). More recently, this transition has been studied by deuterium nuclear magnetic resonance (^{2}H NMR[1]) (Davis et al., 1979; Kang et al., 1979b).

Cronan & Gelmann (1975) and Cronan (1978) have reviewed the numerous studies on the relationship between the physical state of the membranes and their physiological functions. In a recent calorimetric study, Jackson & Cronan (1978) reported that, for wild type *E. coli*, the entire phase-transition region of the extracted lipids is well below the growth temperature (whether the cells were grown at 37 °C or at 25 °C). They also demonstrated by calorimetry on the extracted lipids that certain *E. coli* mutants can grow (at a reduced rate) at a temperature where more than half of the lipids are in the gel phase.

There is some difficulty in estimating the relative fractions of fluid- and gel-phase lipid from calorimetric data in whole membranes because nonlipid components contribute to the thermal properties. The fractions of fluid- and gel-phase lipid in *E. coli* membranes have been measured as a function of temperature by X-ray diffraction (Overath et al., 1975). However, when this method is used, the intensity of the high-angle Bragg peak (at 4.2 Å) of the gel-phase lipids depends on the average size of the gel-phase domains (long-range order) as well as on the orientational (short-range) order of the hydrocarbon chains. Lipid molecules in small spatially ordered domains will not contribute to this X-ray diffraction peak.

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[1] Abbreviations used: ^{2}H NMR, deuterium nuclear magnetic resonance; GLC, gas–liquid chromatography; DEGS, diethylene glycol succinate; KDO, 2-keto-3-deoxyoctonate; LPS, lipopolysaccharide; DPPC, dipalmitoylphosphatidylcholine.

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