Proton and Carbon-13 Nuclear Magnetic Resonance Studies of the Polar Lipids of *Halobacterium halobium*†

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ABSTRACT: The thermotropic behavior and the dynamic properties of the polar lipids of *Halobacterium halobium* were studied by ^1H and ^{13}C NMR. The studies were performed on three different preparations: micellar solutions in deuterated chloroform, multilamellar dispersions in ^2H_2O, and unilamellar vesicles obtained from the latter by ultrasonication. Due to the methyl side groups in the alkyl chains of these lipids, the ^{13}C spectra are highly resolved in particular in the micellar solution, allowing peak assignment of the alkyl chain carbons. Proton and ^{13}C T_1 relaxation and line width measurements were made for all three preparations. In both lamellar dispersions, sharp discontinuities in these parameters were observed around 35 °C. This behavior is attributed to an abrupt change in the dynamics of the lipids within the membrane. The variation of the specific relaxation rate $1/NT_1$ of the ^{13}C nuclei along the hydrocarbon chains of the *H. halobium* lipids exhibits maxima at the tertiary carbons to which the methyl side groups are attached. These maxima are particularly pronounced in the lamellar phases and suggest that the segmental motion ("kink" formation) at the tertiary carbons is hindered by the presence of the methyl groups.

The lipid composition of the purple membrane of *Halobacterium halobium* has been discussed in detail by Oesterhelt (1976) and by Bayley & Morton (1978). It is of considerable interest in view of the well-known function of this membrane as a light-driven proton pump. Purple membrane occurs in insular regions which in some mutants may cover as much as half of the cell membrane surface (Oesterhelt & Stoeckenius, 1973). These regions contain only one protein, the purple pigment bacteriorhodopsin, tightly-packed in a two-dimensional hexagonal crystalline lattice within a lipid matrix (Blaurock & Stoeckenius, 1971). Blaurock (1975) estimated that the composition of isolated purple membrane corresponds to ~10 lipid molecules per single protein molecule and showed the most satisfactory arrangement for the bacteriorhodopsin molecules to be in clusters of three. Simultaneous X-ray diffraction studies by Henderson (1975) arrived at similar conclusions. The most comprehensive studies of the composition of the lipids of halophilic bacteria are of *Halobacterium cutirubrum* (Kushwaha et al., 1975), but preparations from *H. cutirubrum* and *H. halobium* have been found to be essentially identical (Kushwaha et al., 1976).

In whole cell extracts (both red and purple membrane), 90% of the lipids consist of polar phospholipids and glycolipids and about 10% consist of neutral isoprenoid derived lipids (Kates, 1972). The side chains of all their polar lipids are phytanyl groups [3(*R*),7(*R*),11(*R*),15-tetramethylhexadecyl], ether linked to the glycerol moiety (2,3-di-*O*-phytanyl-*sn*-glycerol). (Halobacteria do not synthesize fatty acids.)

The head groups of the polar lipids are different; however, they are all negatively charged (except for minute amounts of uncharged glycolipids). The major phospholipid (65% by weight) was shown to be the diether analogue of 1-*sn*-phosphatidyl-3′-*sn*-glycerol 1′-phosphate (Kates, 1972). Two minor phospholipids (about 4%) were identified as diether analogues of 1-*sn*-phosphatidyl-3′-*sn*-glycerol and of 1-*sn*-phosphatidyl-3′-*sn*-glycerol 1′-sulfate. About 25% of the total polar lipids consist of glycolipids, including diether analogues of 1-(3′-sulfo-*O*-galactosyl)-β(1′–6′)-mannosyl-α(1′–2′)-glycosyl-α(1′–1′)-*sn*-glycerol and the triglycosyldiphytanyl ether derived by desulfation of the previous compound. The lipid composition of the red and purple membrane was found to be very similar except for the sulfated lipid components which were present exclusively in the purple membrane, the red membrane containing two unidentified glycolipids instead (Kates & Kushwaha, 1978).

There are contradictory results concerning the phase transition temperature for the halophilic lipids. Chen et al. (1974) found that the lipids of *H. cutirubrum*, dispersed in water or 2 M NaCl, do not undergo phase transitions above −35 °C. However, in liposomes prepared from unfractionated *H. cutirubrum* lipids, an irreversible thermal transition at ~26 °C was observed by studying the rotational mobility of the fluorescent probe perylene (Lanyi, 1974). Liposomes prepared from polar lipids alone and cell envelopes from *H. cutirubrum* (not containing purple membrane) did not show this transition. Chignell & Chignell (1975), using spin-labeled fatty acids as probes, found a reversible thermal transition at ~29 °C in purple membrane from *H. halobium*, which they attributed to a change in the structure of bacteriorhodopsin since it vanished on cross-linking with glutaraldehyde. Korenstein et al. (1976) determined Arrhenius parameters for the formation and decay of phototransients in suspensions of purple membrane over the range 0–60 °C in both ^1H_2O and ^2H_2O and observed transition points between 25 and 32 °C for the longest lived transients. Simultaneous measurements of microviscosity using DPH showed a transition at ~23 °C [erroneously printed as 30 °C in Korenstein et al. (1976)]. Gupte et al. (1976) also observed a phase transition at about 31 °C in purple membrane from *H. halobium* using a spin-labeled probe, which was shifted to about 22 °C in subbacterial particles. In proton NMR studies, the relaxation time of the two phytanyl methyl groups provides an intrinsic probe for membrane viscosity. Using this approach in vesicles prepared from *H. halobium* lipids, Degani et al. (1978) showed a break in the Arrhenius plot of the proton relaxation times at 31 ± 4 °C. Differential scanning calorimetry has been used by Jackson & Sturtevant (1978) in a study of the phase transitions of purple membrane suspensions. A small endothermic reversible transition was seen at ~80 °C, and a larger one was

† From the Department of Isotope Research (H.D.) and the Department of Membranes Research (A.D. and S.R.C.), The Weizmann Institute of Science, Rehovot, Israel. *Received August 15, 1979.* This study was supported by grants from the U.S.–Israel Binational Science Foundation (BSF), Jerusalem, Israel, from the Israel Commission for Basic Research, from the National Council for Research and Development, Israel, and from the KFA Julich, Germany.

0006-2960/80/0419-1626$01.00/0

seen at 100 °C corresponding to the irreversible denaturation of bacteriorhodopsin. No transition at all, whatever the aqueous medium used, was detected over the entire range from 0 to 75 °C. However, Degani et al. (1978) repeated this study using a different type of calorimeter and observed a small but reproducible transition in the range 27–40 °C.

In the present work we describe an NMR study of the polar lipids extracted from *H. halobium*. We were particularly interested in determining whether a temperature-induced alteration such as a phase transition does occur in the liquid-crystalline phases of the *H. halobium* lipids and to what extent the chain dynamics and structure are affected by the presence of the methyl side groups along the chain.

It is now generally accepted that the so-called gel to liquid-crystalline phase transition is associated with a decrease in the orientational order and a concomitant increase in the segmental motion of the hydrocarbon chains and lateral diffusion of the lipid molecules. These changes manifest themselves in several magnetic resonance properties, including nuclear longitudinal (T_1) and transversal (T_2) relaxation times. In this study we have measured the ^1H and ^{13}C spectra of the *H. halobium* polar lipids in several preparations including a micellar solution in C^2HCl_3, a multilamellar dispersion in ^2H_2O, and a vesicular dispersion obtained from the latter by sonication. The quantities measured were the half-widths of the various ^1H and ^{13}C resonances and the longitudinal relaxation rates of the ^1H resonances as functions of temperature both above and below the phase transition. In addition, we measured at one particular temperature the ^{13}C longitudinal relaxation rates of the various carbons.

The most informative results come from the ^{13}C spectra, since unlike the ^1H case many of the carbons along the alkyl chains give well-resolved peaks whose relaxation times can be accurately determined. This increased resolution allowed us to measure the relaxation profile along the chain and thus to study the effects of small perturbations of the methyl groups on the chain dynamics. The results clearly exhibit a temperature-induced sharp change in the dynamic properties of the lipid chains in the vesicular suspension between 30 and 40 °C. The origin of the change is not yet clear, whether it is a true thermal liquid-crystalline–gel phase or some other structural change such as formation of quasi-crystalline clusters.

From these results it can be seen that the *H. halobium* lipids constitute a unique model system. In addition to its enormous interest from a purely bioenergetic point of view, this system is capable of providing a great deal of information on membrane structure and dynamics not readily obtainable in other systems.

Experimental Section

Extraction of Polar Lipids from H. halobium. Cells were grown aerobically for 4 days in a medium described previously by Eisenbach et al. (1977). The cells were harvested at low speed in a Sharples centrifuge and then washed once with 4 M NaCl and lysed with water in the presence of DNase. The lysate was centrifuged for 15 min at 13000*g* and then for 90 min at 300000*g*. Total lipids were extracted from the resulting pellet suspended in water according to the method of Kushwaha et al. (1975). The polar lipids were then separated by precipitation with acetone (Kushwaha et al., 1975). The precipitation procedure was repeated 3 to 4 times to eliminate all the nonpolar lipids.

Sample Preparation. Solutions of polar lipids in deuterated chloroform were prepared at concentrations of ~75 mg in 1 mL of solvent.

Multilamellar suspensions of the *H. halobium* polar lipids were prepared by dispersing the lipids (70 mg/mL) in a 1 M NaCl solution of ^2H_2O at p^{2}H 7 and vortexing for about 10 min at 40 °C. Such suspensions were stable for several hours.

Vesicles were prepared by sonicating a multilamellar dispersion at 40 °C under nitrogen for 15 min (Heat System W-375 sonifier; $^3/_8$-in. tip; power level 5, pulsed mode; 40% fractional power). The clear sonicated suspensions were then centrifuged for 30 min at 40000*g* and 40 °C, and the zone containing clear supernatant was removed for subsequent studies. All experiments were performed within 72 h after the preparation of the vesicles.

NMR Measurements. Proton and ^{13}C NMR measurements were carried out at 270 and 67.89 MHz, respectively, on a Bruker WH-270 pulse-FT spectrometer equipped with variable temperature accessories which maintain the temperature to ±1.5 °C. The spectrometer was locked on internal solvent deuterium. Typical conditions for ^1H studies were a spectral width of 2.4 kHz using 8K data points in the frequency domain. One to ten transients were needed in order to get good signal to noise ratios. Typical conditions for ^{13}C studies were a spectral width of 7 kHz using 8K data points employing the quadrature detection mode. In most experiments carbon-13 resonances were decoupled with 10-W broad band proton noise decoupling. The number of transients accumulated ranged between 600 and 10000. Longitudinal relaxation rates were measured by the inversion recovery method. For each T_1 of a proton resonance, 12 values were used, providing results accurate within 5%. For ^{13}C T_1 measurements at least four and up to eight values were used, and the resulting accuracy is estimated at ±20%.

Results

^1H Spectra. Proton NMR spectra of the *H. halobium* lipid micelles in C^2HCl_3 as well as vesicles and multilamellar suspensions in H_2O are shown in Figure 1. For comparison, the corresponding spectra of dipalmitoylphosphatidylcholine (DPPC) are shown in Figure 2.

It may be seen that in the *H. halobium* lipids the resonances due to the methyl, methylene, and methine protons as well as those of the polar head group are readily discerned, and there is some structure within the methylene region which is attributed to different CH_2 groups. However, the resolution is not sufficient to resolve the peaks due to individual protons within each group. In all samples the relative intensities of the various signals are approximately given by the relative number of the corresponding protons, indicating that the total spectrum of the alkyl chain is observed.

In both types of lipids the overall line widths of the various bands increase on going from the micellar to the vesicular and multilamellar solutions. However, in the DPPC multilamellar spectrum the broadening is considerably larger than in the corresponding *H. halobium* preparation.

In Figure 3 are plotted the longitudinal relaxation rates of the various protons in the vesicular dispersion of the *H. halobium* lipids as a function of temperature in the range 3–55 °C. Also shown in Figure 3 is the temperature dependence of the methyl resonance line width (at half-maximum intensity). Because of the inhomogeneous broadening of the methylene signals, no line width data for these resonances are given. As may be seen, the longitudinal relaxation rate of the various protons as well as the methyl line width undergoes a conspicuous change at slightly above 30 °C.

^{13}C Spectra. A considerably better resolution of the various chain segments is obtained in the ^{13}C spectrum. Typical spectra of the three preparations of the *H. halobium* lipids

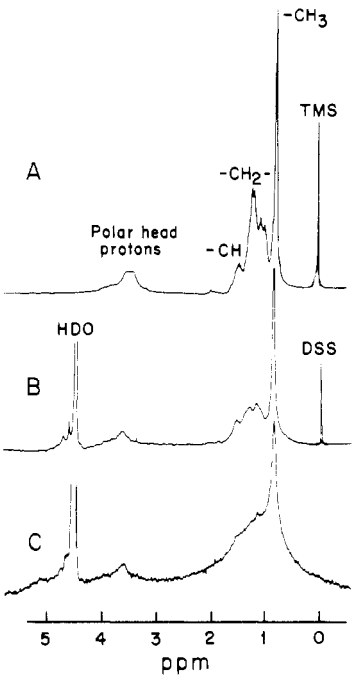

FIGURE 1: ^1H spectra of the polar lipids extracted from *H. halobium*. (A) Lipids dissolved in deuterated chloroform at 28 °C. (B) Vesicle suspension in ^2H_2O containing 1 M NaCl, p^{2}H 7, at 43 °C. (C) Multilamellar suspension in the same solution as (B) at 43 °C. TMS, tetramethylsilane; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

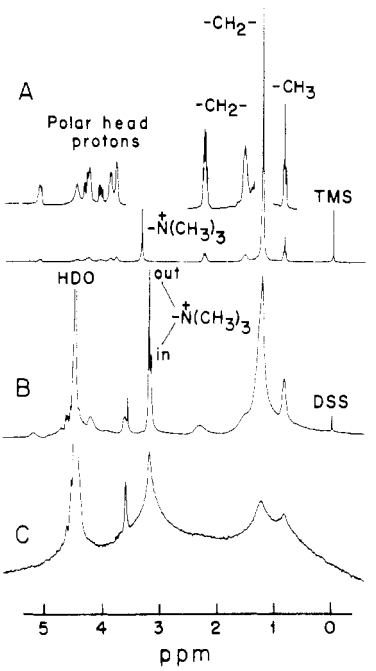

FIGURE 2: ^1H spectra of DPPC (dipalmitoylphosphatidylcholine). (A) Lipids dissolved in deuterated chloroform at 28 °C. (B) Vesicles in ^2H_2O containing 10 mM Tris, p^{2}H 7.7, at 43 °C. (C) Multilamellar suspension in the same solution and conditions as (B) (in trace B "in" and "out" refer to signals due to internal and external interfaces, respectively).

including the peak assignment for both the *H. halobium* lipids and DPPC are shown in Figures 4 and 5, respectively. The signal assignment for DPPC is according to Levine et al. (1972). The assignment for the *H. halobium* lipid spectra was made on the micellar solution. It was based on comparison of proton decoupled and fully coupled spectra and on T_1 measurements to be described below, as well as on comparison

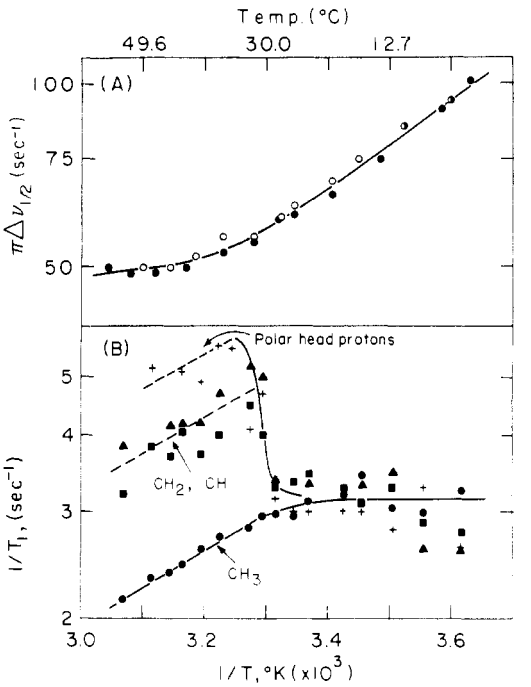

FIGURE 3: Temperature dependence of the proton line width ($\pi\Delta\nu_{1/2}$) and longitudinal relaxation rates ($1/T_1$) in *H. halobium* vesicles as in Figure 1B. (A) Line width of the methyl protons. The full circles refer to vesicles containing 20% by weight of purple membrane. (B) $1/T_1$ for the various protons as indicated in the figure.

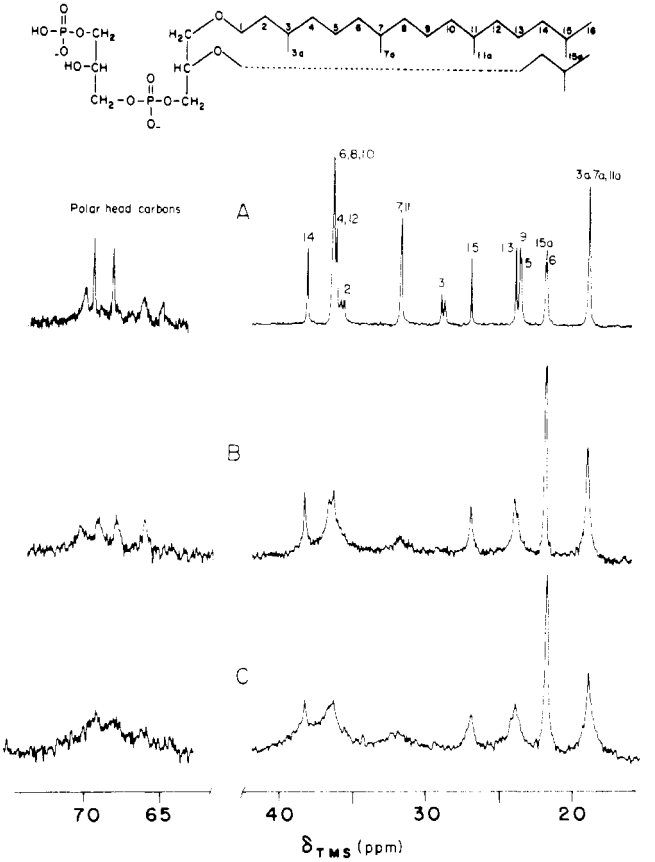

FIGURE 4: Proton decoupled ^{13}C spectra of the *H. halobium* lipids. (A) In deuterated chloroform at 28 °C. (B) Vesicles as in Figure 1B at 43 °C. (C) Multilamellae as in Figure 1C at 43 °C. The structural formula of the dominant polar lipid phosphatidylglycerophosphate together with the numbering convention is also given.

with analogous data for phytol and pristane (Goodman et al., 1973) and the expected peak positions from ^{13}C chemical shift

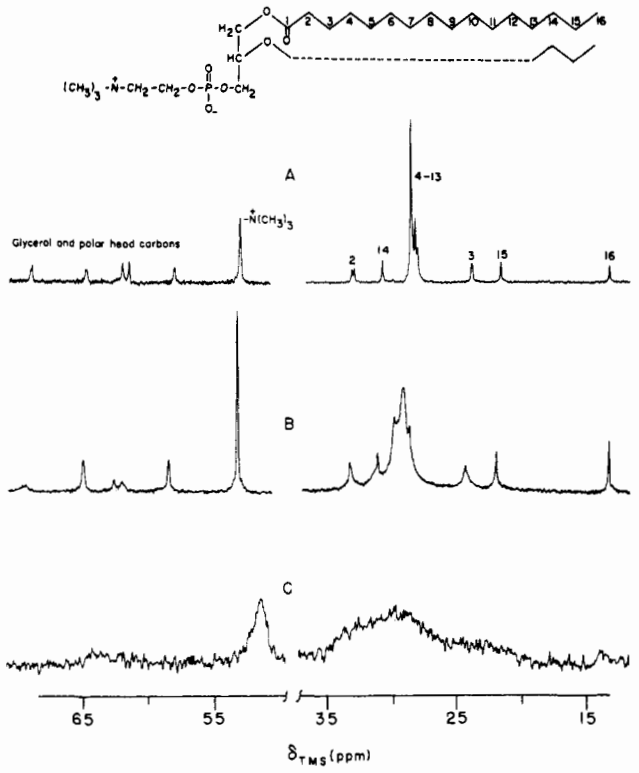

FIGURE 5: Proton decoupled ^{13}C spectra of DPPC. (A) In deuterated chloroform at 28 °C. (B) Vesicles as in Figure 2B at 43 °C. (C) Multilamellae as in Figure 2C at 43 °C.

parameters. There are some special features to note in the high-resolution spectrum of *H. halobium* micellar solution: in particular, the small splitting of the end methyl groups 15a and 16 which results from the optical center at carbon 11 and the doublet splitting of carbons 2 and 3 which we ascribe to the inequivalence of the two phytanyl side chains. These carbon atoms (and carbon 1 which is part of the polar head group band) are nearest to the glycerol moiety and are most sensitive to the inequivalent bonding positions of the side chains. Similar splitting is observed for carbons 2 and 3 of DPPC.

The ^{13}C spectrum of the *H. halobium* polar lipids is considerably more resolved than the corresponding unbranched phospholipid DPPC. The higher resolution of the former lipids is caused by the methyl side chains which induce a shift in the carbon atoms to which they are directly linked as well as in the next nearest neighbors. Thus, instead of a single band corresponding to carbons 4–13 as in DPPC, here many of the peaks are resolved. However, on going from the micellar to the vesicular and multilamellar samples, there is a gradual increase in the line width, except for the end methyl peaks (15a and 16) which are only very little affected (see Figure 4). As a result, some of the resolution is lost and some of the peaks (e.g., the methine carbons) are even broadened beyond detection. The broadening of the various peaks can be estimated from their relative heights with respect to the end methyl signal. Roughly, it may be seen that in the bilayer phases the line width of the methylenes beyond C-12 decreases toward the end methyl group, but it remains approximately constant for the CH_2's below C-12. This trend is retained over a wide temperature range in both liquid-crystalline phases. It is clearly seen in Figure 6 where a number of spectra at different temperatures are depicted and in Figure 7 which shows a plot of the resonance line widths as a function of temperature. It may also be seen that the line widths of all the ^{13}C resonances

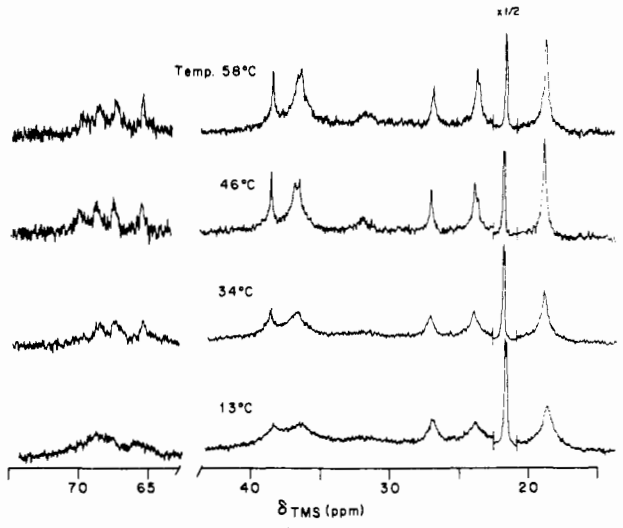

FIGURE 6: Proton decoupled ^{13}C spectra of *H. halobium* vesicles at various temperatures. Vesicle suspension is the same as that in Figure 1B.

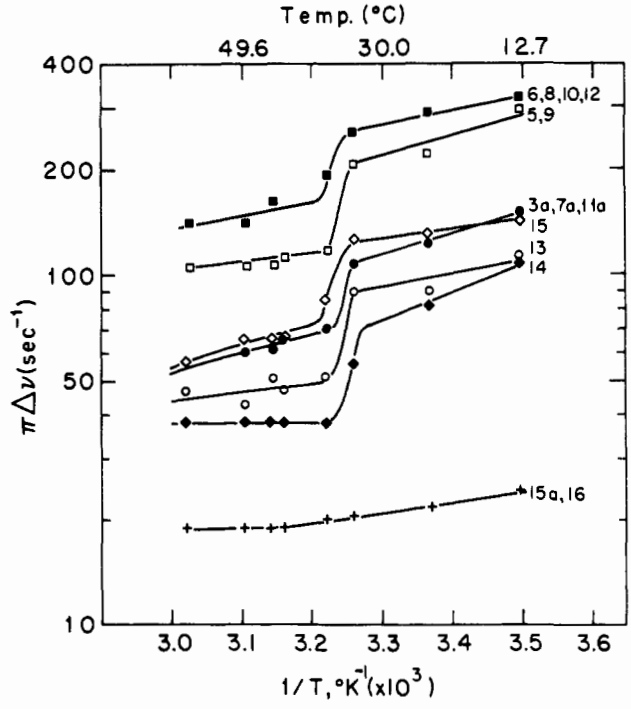

FIGURE 7: The temperature dependence of the ^{13}C line width of the chain carbons in *H. halobium* vesicles.

(except for the end methyls) exhibit a discontinuity between 30 and 40 °C.

As for the proton case, the ^{13}C spectrum of the multilamellar preparation of DPPC gives a much broader signal than the corresponding *H. halobium* lipids. It should, however, be noted that the ^{13}C spectrum of DPPC was accumulated for about 2 h during which time some phase separation may have occurred.

Finally, we present ^{13}C T_1 relaxation data for the alkyl chain carbons for all three preparations. The results are summarized in Table I and plotted in Figure 8 as a function of the carbon positions along the chain. It may be seen that at the tertiary carbons there is a peaking of the $1/NT_1$ plots. It should be noted that the exact periodic regularity of the curves is in part due to the fact that some of the resonances are not resolved, e.g., carbons 7, 11 and 5, 9 and 6, 8, 10, 12. However, the T_1 relaxation plots of these signals showed within experimental

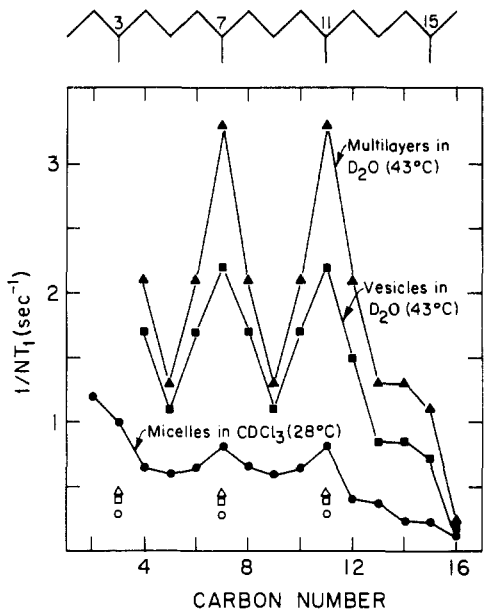

FIGURE 8: ^{13}C spin–lattice relaxation rates for the chain carbons of *H. halobium* lipids. Open symbols refer to the relaxation of the branched methyl carbons at the indicated position. It should be noted that an increase in the quantity $1/NT_1$ reflects a decrease in the motional freedom.

Table I: ^{13}C Chemical Shifts and Spin-Lattice Relaxation Rates within the Chains of the *H. halobium* Lipids

carbon no.	δ_{Me_4Si} in C^2HCl_3, 28 °C (ppm)	$1/T_1$ (s^{-1}) C^2HCl_3, 28 °C	vesicles, 43 °C	multilamellae, 43 °C
16, 15a	21.63, 21.72	0.42	0.58	0.74
15	26.97	0.23	0.72	1.1
14	38.39	0.46	1.7	2.7
13	23.82	0.74	1.7	2.6
12, 4	36.34	0.80, 1.3	3.0, 3.5	4.2
11, 7	31.83	0.82	2.2	3.3
6, 8, 10	36.54	1.3	3.5	4.2
9, 5	23.54	1.2	2.1	2.6
3	28.86, 28.95	1.0		
2	36.08, 35.89	2.3		
11a, 7a, 3a	18.71	0.86	1.2	1.3

accuracy a single exponential decay, indicating that all carbons in a given group have similar relaxation times.

Discussion

Thermotropic Behavior. The main result of the previous section is the observation of an abrupt change in the motional freedom of the *H. halobium* lipids within the bilayer between 30 and 40 °C. This is exhibited by several phenomena: the discontinuity in the ^{13}C line widths of the various lipid chain carbons (Figure 7), the discontinuity in the ^1H longitudinal relaxation rates of the methylene, methine, and polar head protons, and the breaks in the plots of the methyl proton relaxation rates (Figure 3). These changes were studied primarily in the vesicular preparations but were also observed in the multilamellar suspensions of the *H. halobium* lipids. It should be added that in general there is no drastic difference between the NMR spectra of the two lamellar preparations. The line widths and longitudinal relaxation rates in the multilamellar dispersion are about twice as large as in the vesicles, but no "disappearance" of signal intensity or appearance of a broad background component, as is found in the DPPC multilamellar preparation, is observed in the *H. halobium* case.

As previously mentioned, the temperature-induced alterations in the NMR motional parameters may be ascribed to a true liquid-crystalline–gel phase transition. However, other structural changes that may occur in the liquid-crystalline phase such as formation of quasi-crystalline clusters (Lee et al., 1974; Wunderlich et al., 1975) cannot be excluded.

In the following, we interpret the relaxation data in terms of the molecular dynamics in the various *H. halobrium* lipid preparations.

Relaxation Data. The quantitative interpretation of the ^1H relaxation data is quite complicated because several mechanisms can contribute to it, including intra- and intermolecular dipolar interactions as well as spin diffusion. In both phases we find $1/T_2 >> 1/T_1$, and this will be discussed below for the ^{13}C case in terms of a slow motion process that contributes to the line width. The most pronounced effect in the proton results is, however, the temperature dependence of the T_1 relaxation (Figure 3). While the results for the methyl protons change continuously on going from the gel to the liquid-crystalline phase, the relaxation rates for the CH_2, CH, and polar head group protons change abruptly at the transition region. In the high-temperature phase, $1/T_1$ of these protons is about twice as large as that of the methyl protons while in the low-temperature phase they all become equal. These results suggest that there might be a change in the dominant relaxation mechanism of the ^1H nuclei in the two phases. For example, it is possible that the dominant mechanism changes from dipolar interaction in the high-temperature phase to spin diffusion in the low-temperature one. Detailed analysis of these data must await more extensive measurement and theoretical computation.

The interpretation of the ^{13}C relaxation data is relatively simple because the ^{13}C spectra are highly resolved and the relaxation rates can usually be interpreted in terms of a single mechanism, e.g., the intramolecular dipolar interaction with the directly bonded protons. We have measured the Overhauser effect and determined an enhancement factor of ~ 3 for all carbons in both the micellar and vesicular systems. This confirms that in our system too the ^{13}C relaxation is dominated by the intramolecular C–H dipolar interaction.

The interpretation of the relaxation data follows the same lines as those of Gent & Prestegard (1977) and involves only order of magnitude considerations. We note that for all ^{13}C lines the transverse relaxation rate $1/T_2$ (calculated from the line width by $1/T_2 = \pi\Delta\nu$) is considerably larger than the longitudinal relaxation rate ($1/T_1$) and that both rates for all lines decrease with increasing temperature. This behavior, found also for other phospholipids, can be explained in terms of a model in which the C–H dipolar interaction is modulated by two processes: one process with a short correlation time τ_D, for which $\omega\tau_D << 1$ (where ω is ^{13}C Larmor frequency), and a second process with a long correlation time τ_C, for which $\omega\tau_C >> 1$. The first correlation time may be associated with the modulation of the C–H dipolar interaction by the segmental reorientation of the CH_2 fragments of the alkyl chains, for example, by β-coupled gauche isomerization ("kink" formation). This process is responsible for the ^{13}C T_1 relaxation, and its correlation time is related to T_1 by

$$\frac{1}{NT_1} \approx \frac{4}{3}\langle H'd^2\rangle\tau_D$$

where N is the number of protons bonded to the ^{13}C nucleus

$$\langle H'd^2\rangle = \langle Hd^2\rangle - \langle \bar{H}d^2\rangle$$

and $\langle Hd^2 \rangle$ is the average of the square of the full ^{13}C–^{1}H dipolar interaction ($\sim 3.05 \times 10^{10}$ s^{-2}) while $\langle \bar{H}d^2 \rangle$ is the average of that part of the dipolar interaction not averaged out by the segmental motion. This latter quantity is not zero because the segmental motion is not isotropic. It has been estimated by Gent & Prestegard (1977) on the basis of a β-coupled isomerization mechanism that $\langle \bar{H}d^2 \rangle \sim [(1/4) \cos^4 (2\pi/3)] x \langle Hd^2 \rangle \sim 4.8 \times 10^8$ s^{-2}. The T_2 relaxation is mainly controlled by the slow modulation of this residual dipolar relaxation, for example, by reorientation of the whole vesicles, lateral diffusion around the vesicle surface, or chain wagging. The correlation time for the slow motion is related to T_2 by

$$\frac{1}{NT_2} \approx \frac{4}{15} \langle Hd^2 \rangle \tau_C$$

Thus, using the results in Figures 7 and 8, we calculate for the high-temperature liquid-crystalline phase (at $\sim$43 °C) the following (average) correlation times:

$$\tau_D \sim 3 \times 10^{-11} \text{ s} \qquad \tau_C \sim 5 \times 10^{-7} \text{ s}$$

These results are similar to those found in other phospholipid membranes (Gent & Prestegard, 1977). From the line width data in Figure 7, it follows that at the transition τ_C changes by about a factor of 2. However, the end methyl carbons (15a and 16) are not affected by this transition because of the very fast rotations of the end segment. In general, the methylenes close to the end methyl have narrower line widths, apparently because $\langle \bar{H}^2 \rangle$ decreases toward the chain's end.

Finally, we refer to the peaking of the $1/NT_1$ results at the methine carbons (Figure 8). This effect is most pronounced in the vesicular and multilamellar preparation of the *H. halobium* lipids. For the sake of comparison, in DPPC $1/NT_1$ for carbons 4–13 is essentially constant (Metcalfe et al., 1971). Since T_1 is predominantly determined by τ_D, it may be concluded that the kink formation at the methine carbons in the ordered phases is hindered by the presence of the methyl side chains. The $1/NT_1$ peaking of the methine carbons in the micellar preparation is much smaller and could be due to a small contribution to $1/T_1$ from the next neighbor methyl protons.

Conclusions

It is clear from the above studies that the lipids of halobacteria undergo an abrupt change in their motional freedom, which in the case of *H. halobium* is at $\sim$35 °C. This change may be correlated with the observed break in the kinetics of the phototransients of bacteriorhodopsin at the same temperature range. The correlation between the protein activity and the lipid dynamics probably reflects cooperativity in lipid–protein interaction.

Lipids from halobacteria constitute an experimental model capable of yielding a large amount of information. The presence of the methyl groups causes restricted local segmental motion, but whole chain motion is unaffected. In fact, relative to DPPC, whole chain motion may be enhanced—particularly at low temperatures.

Acknowledgments

We are grateful to Professor Z. Luz for his help and stimulating discussions throughout this work and for his critical review of the manuscript.

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