Biochimica et Biophysica Acta, 688 (1982) 622–636
Elsevier Biomedical Press

BBA 71214

A ^{2}H-NMR STUDY OF *ACHOLEPLASMA LAIDLAWII* MEMBRANES HIGHLY ENRICHED IN MYRISTIC ACID *

HAROLD C. JARRELL, KEITH W. BUTLER, R. ANDREW BYRD, ROXANNE DESLAURIERS, IRENA EKIEL and IAN C.P. SMITH

Division of Biological Sciences, National Research Council of Canada, Ottawa K1A 0R6 (Canada)

(Received December 22nd, 1981)

Key words: Phase transition; ^{2}H-NMR; Myristic acid; Membrane structure; (A. laidlawii)

Myristic acid specifically deuterated at several positions along the acyl chain was biosynthetically incorporated into the membrane lipids of *Acholeplasma laidlawii* B to the level of ⩾90%. ^{2}H-NMR was used to study the molecular order and lipid phase composition of the membranes as a function of temperature. Isolated membranes and intact cells give rise to similar ^{2}H spectra. Below 25°C the spectra exhibit a broad gel phase component which at 0°C reaches the rigid limit value expected for an immobilized methylene group. Spectral moments were used to determine the relative amounts of gel and liquid crystalline phase lipids throughout the gel-liquid crystal phase transition. The results indicate that at the growth temperature (37 or 30°C) the *A. laidlawii* B membrane lipids are ~85–90% in the gel state, and that protein has little effect on lipid order of the liquid crystalline lipid, but leads to an increase in the linewidth by approx. 20%.

Introduction

In order to understand membrane function it is essential to have detailed knowledge of the structure and dynamics of the lipid molecules, in particular of the hydrocarbon chains which provide the hydrophobic environment for various processes. ^{2}H-labelled lipids provide non-perturbing probes whose NMR spectra are sensitive to molecular structure and dynamics within membranes. Recently, in studies of *A. laidlawii* [1–6] and *Escherichia coli* membranes [6–10] the technique has been used to elucidate membrane organization. *A. laidlawii* B membranes in the fluid phase exhibit a hydrocarbon flexibility gradient, or order-position profile, which is similar to that of multilamellar dispersions of lipids [1,3,4]. In addition, ^{2}H-NMR studies of *E. coli* [9,10] and *A. laidlawii* B [2,3] have provided information about membrane lipids in the gel state.

An important aspect of structural studies, which has been extensively investigated, is the temperature dependence of molecular order in membranes. The membrane lipids of *E. coli* and *A. laidlawii* exhibit relatively-well defined liquid crystal to gel state transitions, in spite of the heterogeneity of lipid headgroup and fatty acyl chains, and the presence of protein. There is evidence that the degree of membrane fluidity gives rise to a number of important effects. The rates of ATP hydrolysis by membrane-bound ATPase [11], and of glucose transport in *A. laidlawii* [12], have been reported to depend upon the phase behaviour of the membrane lipids. It is well established that *A. laidlawii* growth rates are influenced by the phase composition of its membrane lipids [13], and it will not grow when all of the lipids are in the gel state. Another significant effect of temperature is that cells will change the composition of the acyl chains

* N.R.C.C. Publication number 20220.

and alter the relative amounts of the various lipid headgroup classes [14–16] to adapt to their environment.

Since *A. laidlawii* B readily incorporates exogenous ^{2}H-labelled fatty acids into its plasma membrane, the organism is ideal for studying structural and dynamical properties of an intact biological membrane by ^{2}H-NMR. However, due to various factors the fatty acid composition of the membrane may be variable even when only one exogenous fatty acid is provided. As a result, quantitative comparisons of ^{2}H-NMR data between different growths are difficult. Recently, *A. laidlawii* B membranes which contain essentially one fatty acid (approx. 97%) have been obtained when the organism is cultured in the presence of avidin, an inhibitor of endogeneous fatty acid synthesis [14]. As a result, the sharpness and midpoint temperature of the gel to liquid crystalline phase transition may be controlled. The individual lipid classes present in *A. laidlawii* have been shown to exhibit differing phase behaviour [15,17,18] and widely differing transition temperatures for the same fatty acid [14]. Also, in the case of membranes made homogeneous in fatty acid, the ratio of the constituent headgroup classes has been shown to vary over a wide range [14]. Hence, membranes made homogeneous in fatty acid may permit the detection of subtle effects on the structure and dynamics of acyl chains arising from lipid-protein interaction or headgroup variations which might be obscured by the effects of fatty acid chain-length heterogeneity.

In the present study ^{2}H-NMR has been used to study in detail membranes of *A. laidlawii* B made homogeneous in myristic acid. The effect of growth temperature on membrane organization is explored.

Materials and Methods

[14-$^2H_3$]- and perdeuterated myristic acid were obtained from Larodan Lipids, Malmö, Sweden. (2-$^2H_2$)-labelled fatty acids were prepared by α-exchange [19]. [4-$^2H_2$]Myristic acid was prepared by malonate extension of [2-$^2H_2$]lauryl mesylate [19,20].

Acholeplasma laidlawii B was grown at 37 or 30°C as described previously [4], except that avidin (grade II, Sigma Chem. Co., St. Louis, MO) was added to the growth medium at a level of 25 units/litre. Plasma membranes were prepared as described earlier [21]. The acyl chain composition was determined by gas-liquid chromatography (GLC) of the methyl esters [21] and is expressed in mol% (Table I). Lipid extracts were obtained by the method of Wells and Dittmer [22].

The lipid headgroup distribution was analyzed by applying the total lipids to $100 \times 50 \times 0.5$ mm plates of Silica gel G which were developed with chloroform/methanol/water (65 : 25 : 4, v/v). After drying, the plates were briefly exposed to I_2 vapour until the lipid spots were lightly stained. Lipid spots of interest were scraped from the plates and then subjected directly to methyl ester analysis in the presence of a known amount of standard fatty acid. The relative ratio of lipid head groups was determined as described elsewhere [14] and are given in Table I.

The NMR membrane samples consisted of freeze-dried membrane hydrated with deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a weight ratio of about 1:3. Intact cells were washed twice, and the NMR samples prepared with a wash solution [21] prepared with deuterium-depleted water. Cells were checked microscopically after the NMR experiments and exhibited little cell lysis. NMR samples of the lipid extracts were prepared by hydrating the lipids with deuterium-depleted water (1:3, w/w) and repeatedly heating to 45°C and freeze-thawing until the sample was homogeneous.

Calorimetry was performed on a Microcal MC-1 differential scanning calorimeter (DSC) with a temperature scanning rate of 1.0 degree Celsius/min. The gel to liquid crystal transition of fully hydrated dipalmitoylphosphatidylcholine was used to calibrate the temperature scale. Samples weighed approx. 10 mg and were dispersed in 1.5 ml water.

^{2}H NMR spectra were obtained at 46.063 MHz with a Bruker CXP-300 spectrometer using a home-built probe and at 30.7 MHz with a home-built spectrometer (Byrd, R.A., unpublished data). Spectra were obtained using the quadrupole echo technique [23] with phase alteration on the pulse applied along the *x*-direction. The experiments were done on resonance with the phase of the

TABLE I

FATTY ACID COMPOSITION AND HEADGROUP ANALYSIS OF THE TOTAL LIPIDS OF *ACHOLEPLASMA LAIDLAWII* GROWN ON SPECIFICALLY DEUTERATED MYRISTIC ACID (14:0)

MG, monoglucosyldiacylglycerol; DG, diglucosyldiacylglycerol; PG, phosphatidylglycerol.

Labelled carbon atom	Growth temperature (°C)	Yield of freeze-dried membranes (mg)	Percent 14:0	Ratio of MG/DG	Ratio of (MG+DG)/PG
2	37	200	91	1.9±0.2	–
4	37	140	90	1.4±0.1	3.5±0.3
14	37	200	90	0.9±0.1	–
Perdeutero	30	90	92	0.2±0.3	3.7±0.2
Control [a]	37	960	15	1.7±0.2	2.4±0.1

[a] Grown in the absence of avidin and exogeneous fatty acid.

spectrometer reference signal adjusted to give complete absorption signals without the need for any post-Fourier transform phase correction [23]. The sample was enclosed in a glass dewar and the temperature was electronically regulated to within approx. ±0.5°C. After a temperature change the sample was allowed 15 min to come to equilibrium. The temperature gradient across the sample was measured to be ≤0.1°C.

Spectral moments were measured as described previously [24]. The accuracy of the lipid phase fractions calculated for a given temperature was estimated by adding normalized experimental spectra representing pure liquid crystalline and gel phase according to the calculated ratios, and comparing the composite spectrum to the corresponding experimental spectrum [24].

The 90°-oriented sample ^{2}H-NMR spectrum, 'de-Paked', was calculated from the experimental powder spectrum as described elsewhere [25]. Quadrupolar splittings were determined directly from the 'de-Paked' spectrum and linewidths at half-height were calculated by fitting a Gaussian lineshape to the oriented spectrum (Rance, M., Smith, I.C.P. and Jarrell, H.C., in preparation). Simulated spectra were calculated using the experimentally determined quadrupolar splitting, linewidths and an angular-dependent Gaussian lineshape function as described elsewhere (Rance, M., Smith, I.C.P. and Jarrell, H.C., in preparation). 'De-Paked' and theoretical spectra were calculated on a Nicolet NIC-1280 data system.

Results and Discussion

^{2}H-NMR spectra of intact cells vs. isolated membranes

Measurements on *E. coli* [9] and *A. laidlawii* B [6] have determined that lyophilization of the membranes has no effect on the ^{2}H-NMR spectra. However, it is not clear that isolated membranes and intact cells would give rise to identical spectra, a condition which is necessary in order to relate observations made on isolated membranes with membranes of viable cells. Fig. 1 shows ^{2}H-NMR spectra of intact cells and derived membranes of *A. laidlawii* B grown at 37°C on medium supplemented with [14-$^2H_3$]myristic acid in the presence of avidin; the membranes contain 77% myristic acid. At 39°C cells and membranes exhibit very similar spectra, characteristic of only the liquid crystalline phase; both fresh and lyophilized membranes give a calorimetric transition between approx. 24 and 44°C with a midpoint of approx. 36°C. At 32°C both cells and membranes again exhibit similar spectra in which both gel and liquid crystalline phases coexist. The apparent differences in the relative amounts of gel and liquid crystalline phase may be readily explained by the fact that spectra of the membranes are fully relaxed with respect to T_1 while those of the cells are not * (the gel phase spectra have a shorter relaxa-

* In order to avoid cell lysis, spectra were acquired as quickly as possible.

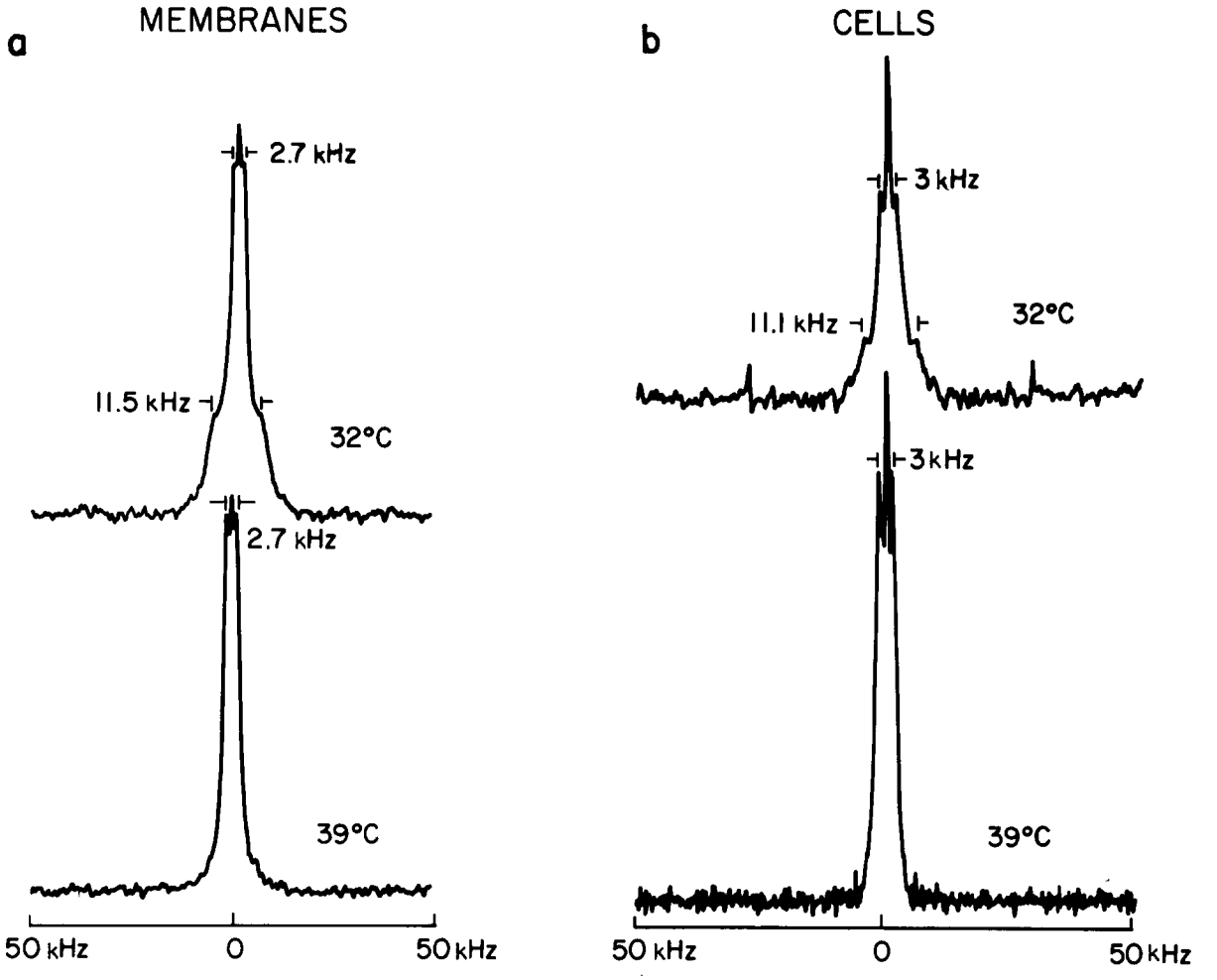

Fig. 1. Temperature dependence of ^{2}H-NMR spectra (a) at 46.063 MHz of membranes containing [14-$^2H_3$]myristic acid (14:0). Spectra acquired using the quadrupolar echo technique and 4.5 μs (90°) pulses, 60 μs pulse separation, 1.2 s recycle time, 250 kHz spectral width and 3000 accumulations; (b) at 30.7 MHz of *A. laidlawii* B cells containing [14-$^2H_3$] 14:0. Spectra were acquired as in (a) with 6 μs (90°) pulses, 60 μs pulse separation, 0.2 s recycle time and 2000 accumulations.

tion time (vide infra). The peak separations in the liquid crystalline and the gel-phase spectra are, within experimental error, the same for isolated and cell membranes (Fig. 1).

The similar molecular ordering and phase behaviour of both systems indicate that conclusions about the organization of isolated membranes are relevant for intact cells.

^{2}H-NMR spectra of membranes homogeneous in myristic acid

Growth at 37°C. Figs. 2–4 show the temperature dependence of membranes containing ($\geq$90%) [2-$^2H_2$]-, [4-$^2H_2$]- and [14-$^2H_3$]myristic acid prepared from cells grown at the optimal growth temperature, 37°C. The three membranes exhibit calorimetric phase transitions which are approx. 15°C in width and have a T_c of approx. 39 $\pm$2°C, where T_c is the phase transition midpoint as defined in Ref. 14. In the case of membranes containing [2-$^2H_2$]myristic acid, only the liquid crystalline phase is present at 45°C (Fig. 2). The oriented spectrum (90° orientation), 'de-Paked', was calculated [25] from the spectrum at 45°C and quadrupolar splittings of 17.6, 23.0 and 25.0 kHz were measured. At 35°C both gel and liquid crys-

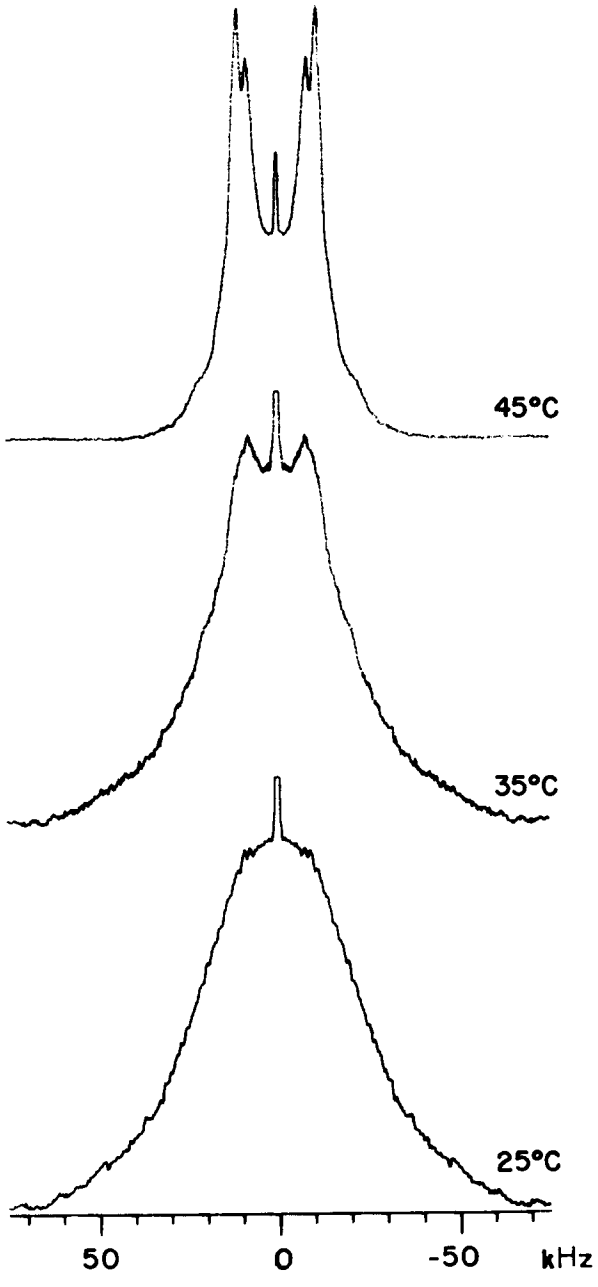

Fig. 2. Temperature dependence of 46.063 MHz spectra of *A. laidlawii* B membranes containing [2-$^2H_2$] 14:0. Spectra acquired as in Fig. 1 with 4.5 μs (90°) pulses, 60 μs pulse separation, 250 kHz spectral width, 0.2 s recycle time and 20000 accumulations.

talline phases coexist. Below 30°C only gel phase lipid is present. The multiple splitting pattern has been well established in a number of membrane systems [1,4,8,27,28]. Recently, the C-2 position of the *sn*-2 chain has been shown conclusively to give rise to two quadrupolar splittings as a result of the magnetic non-equivalence* of the deuterons [29]; the third splitting arises from the equivalent deuterons at C-2 of the *sn*-1 chain.

Although the quadrupolar splittings observed at positions along the chain are similar to those of dimyristoylphosphatidylcholine (see below), there is a significant difference for the 2′ position of the *sn*-2 chain. In the model system splittings of 12 and approx. 18 kHz were reported at 25°C [28] which increased in the presence of added protein [30]. In *A. laidlawii* membranes (45°, Fig. 2) the splittings associated with C-2′ of the *sn*-2 chain have increased by approx. 5 kHz relative to those of the model system. A similar finding in *A. laidlawii* B membranes containing oleic acid has recently been reported [4]; a 5 kHz increase in the quadrupolar splitting relative to those of the corresponding model system was observed. The difference in the splittings observed for *A. laidlawii* B and model membranes were suggested to arise not from the inhomogeneity of the headgroup but from a protein-induced change in the conformation of the lipid molecule in the region of the 2′ position of the *sn*-2 chain. The conformational change was also suggested to be similar from one system to another. However, the extracted lipids from membranes containing [2-$^2H_2$]myristic acid give rise to spectra having quadrupolar splittings (determined from oriented spectra as described above) which are essentially the same, 18, 23 and 26 kHz, as those observed for the membranes. Although the ratios of the headgroup classes in the present study (Table I) are probably different from those reported in the other study [4], it seems that the differences between the quadrupolar splittings of the deuterons at the C-2 positions in *A. laidlawii* membranes and phosphatidylcholines arise from the differences in the headgroups and not from the presence of protein. A slight dependence of the splittings at C-2 on headgroup has been reported

* A similar observation of the magnetic non-equivalence of deuterons has been observed at a different position of a cyclopropane-containing fatty acid in the membranes of *A. laidlawii* B (Jarrell, H.C. and Smith, I.C.P., unpublished data).

in phospholipids [31]. ^{2}H-NMR studies on the individual lipid species would be useful in assigning the effects of the headgroups on the quadrupolar splitting at the C-2 positions of the acyl chains.

The C-4 position exhibits, in the liquid crystalline phase, a peak separation of 29 kHz; from the first spectral moment a quadrupolar splitting of 31.7 kHz is calculated, which is comparable to a value of 31 ± 0.6 kHz reported previously for a similar system [6]. The quadrupolar splitting of the liquid crystalline component remains, within experimental error, constant throughout the phase transition (Fig. 3). At 25°C only a trace of liquid crystalline phase is evident. As the temperature is lowered, spectra attributable to increasingly more rigid lipid are observed until at 0°C essentially no motion at the 4 position is detectable (Fig. 3), with a peak separation of approx. 114 kHz. The spectrum at 0°C lacks any central component, suggesting that the presence of protein does not cause any detectable disruption of the lipid packing. The latter result is noteworthy in that recent studies with reconsituted systems have demonstrated that in the gel state the lipid is disorganized by the presence of protein [32,33]. The absence of such an effect in the 0°C spectrum of membranes containing [4-$^2H_2$]myristic acid may reflect the lower concentration of protein in the membrane, as well as a generally weaker protein-lipid interaction in comparison with the reconstituted systems. Fig. 3e also shows a spectrum at 0°C of *A. laidlawii* B membranes which were grown on [12-$^2H_2$]-palmitic acid (approx. 67% of the total fatty acid) and which had a phase transition between 25 and 45°C. Again most of the spectral intensity is attributable to relatively rigid lipid, with a peak separation of approx. 111 kHz. However, there is a central component which indicates that there is a small fraction of more fluid lipid and suggests that chain-length heterogeneity perturbs the gel state lipid more than does the presence of protein.

In the case of the terminal methyl group of the acyl chain, temperature-dependent spectra (Fig. 4) again show a coexistence of two phases between 30 and 46°C. Below approx. 30°C only gel phase lipid is present. On further cooling the separation of the shoulders increases from approx. 11.5 kHz at 25°C to 14 kHz at 5°C. Interestingly a central narrow component, other than water, persists even at 5°C. The extracted lipids exhibit a liquid crystal type spectrum at 46°C which has a quadrupolar splitting of 3.6 kHz; on cooling to 5°C a gel phase type spectrum with shoulders at ± 7 kHz is obtained which distinctly lacks the narrow central component observed for the membranes. The small quadrupolar splitting observed at 5°C, indicates very considerable disorder and mobility at this position, in contrast to the behaviour at the 2- and 4-positions. A similar result has been obtained with membranes containing pentadecanoic acid (Jarrell, H.C., Butler, K.W. and Smith, I.C.P., unpublished data). Oriented spectra were calculated for the 46°C spectra. A quadrupolar splitting of 3.8 kHz and a associated linewidth of 1.2 kHz were determined for the membranes while the extracted lipids gave rise to a splitting of 3.6 kHz and a linewidth of 1.0 kHz (Fig. 4e). Theoretical spectra were calculated using the determined splittings and linewidths, a Gaussian lineshape function and an angular dependence of the linewidth (Rance, M., Smith, I.C.P. and Jarrell, H.C., in preparation), and were found to give good agreement with the experimental spectra (not shown). In another study [6] on *A. laidlawii* B membranes, the presence of proteins was reported to have essentially no effect on the quadrupolar splitting for all of the acyl chain positions and not to cause any detectable line-broadening effects. In the present study, although the presence of protein does not appear to have any significant effect on the quadrupolar splitting observed for membranes relative to those of the extracted lipids in the liquid crystalline phase, the linewidths appear to increase by approx. 20%. A similar increase in linewidth has been observed for spectra obtained from membranes containing pentadecanoic acid relative to the spectra of the extracted lipids (Jarrell, H.C., Butler, K.W. and Smith, I.C.P., unpublished data). Recent studies [31–35] have also concluded that the presence of protein causes line-broadening effects for lipids in the liquid crystalline phase. In one case [33] the origin of the broadening effect was suggested to arise from the inherent heterogeneity of the system, so that a distribution of quadrupolar splittings occurred and was manifested as an inhomogeneous broadening of the ^{2}H-NMR lines. In the case of the lipids in *A.*

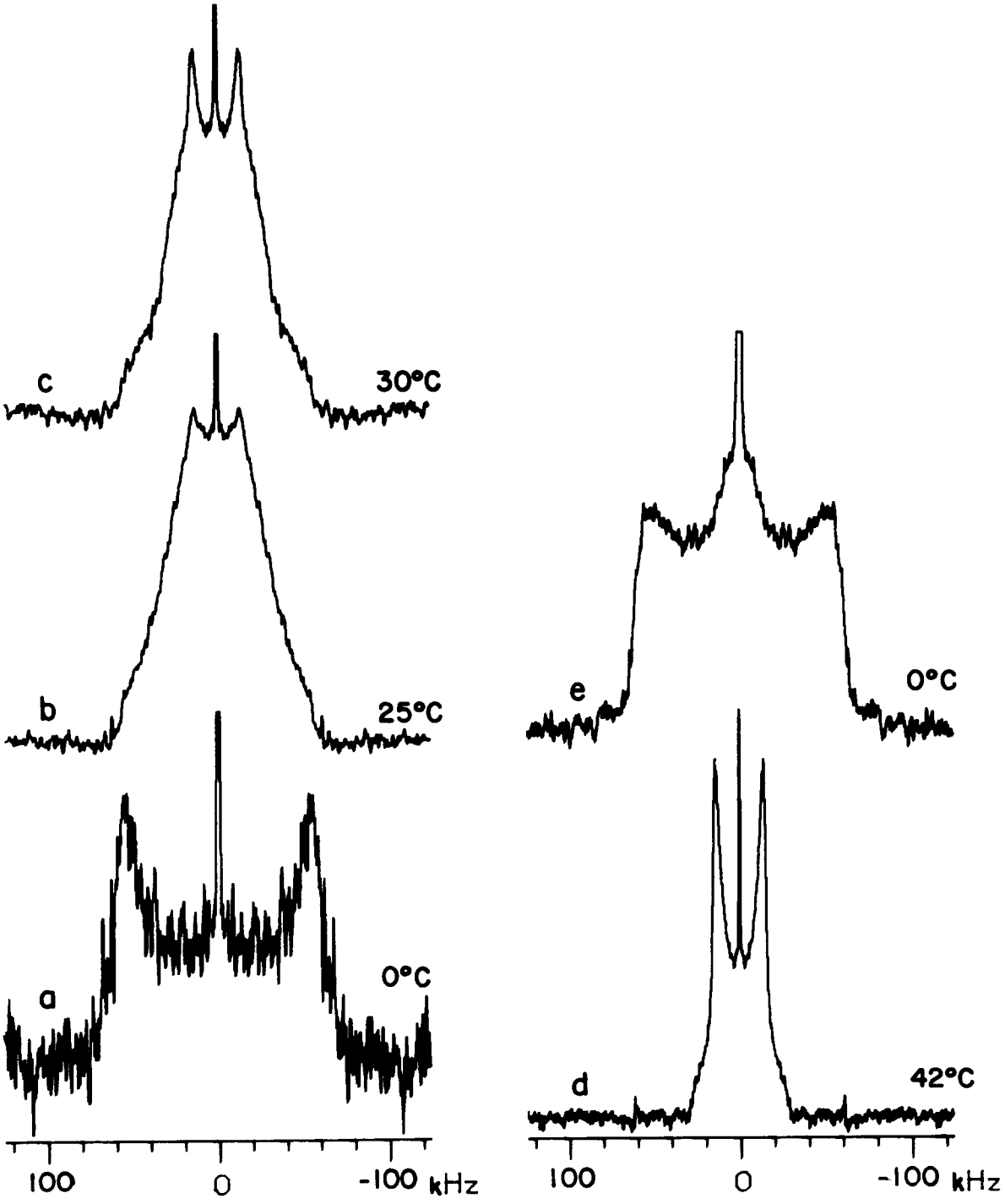

Fig. 3. (a)–(d) Temperature dependence of ^{2}H-NMR spetra of membranes containing [4-$^2H_2$] 14:0. Spectra were acquired as in Fig. 1 with 500 kHz spectral width, 2.25 μs (45°) pulses, 0.2 s recycle time and 160000 accumulations. (e) Membranes containing [12-$^2H_2$] 16:0 (67%). Spectra acquired as in (a)–(d) with 4.5 μs (90°) pulses, and 150000 accumulations.

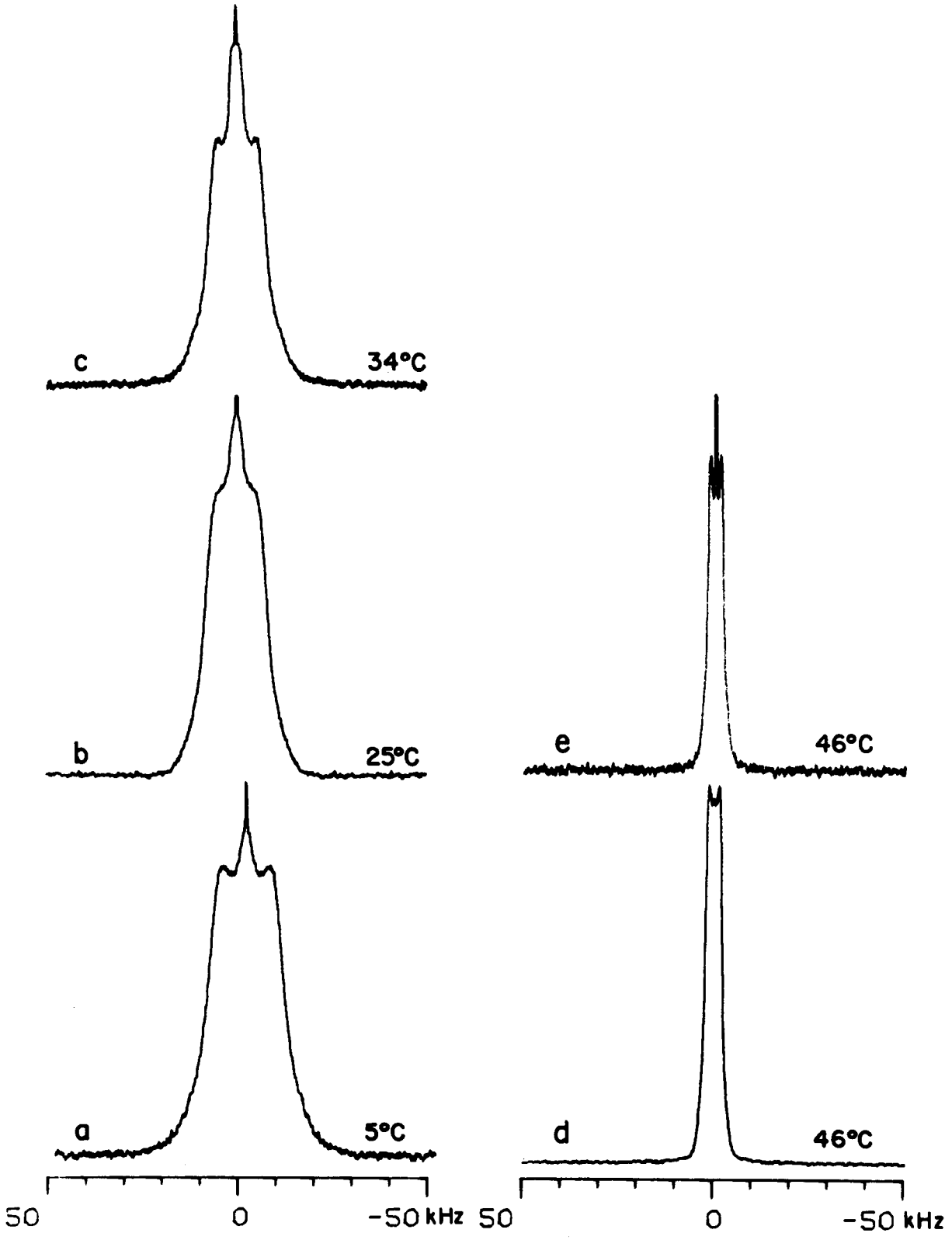

Fig. 4. Temperature dependence of ^{2}H-NMR spectra of, (a)–(d), membranes, and (e), extracted lipids containing [14-$^2H_3$] 14:0. Spectra were acquired as in Fig. 2 with 1.2 s recycle time and 3600 accumulations.

laidlawii membranes the precise source of the line-broadening effect is uncertain; preliminary results indicate that the broadening is a motional effect.

Temperature dependence of the moments of spectra

As has been pointed out previously [2–4,9,10,26] spectral moments provide a method of analyzing the temperature dependence of the quadrupolar powder spectra and provide a useful monitor of the phase transition as well as further insight into the motional behaviour responsible for the spectra. The first moment M_1, is proportional to the mean C-^{2}H bond order parameter, $\langle S_{C^2H} \rangle$, while M_2 is proportional to $\langle S^2_{C^2H} \rangle$. Fig. 5a shows the variation of the first moment with temperature while Fig. 5b shows the corresponding variation of M_2 for membranes containing [4-$^2H_2$]- and [14-$^2H_3$]-myristic acid. Both positions show a sharp increase in M_1 and M_2 as the temperature is decreased through the phase transition, 46–25°C, indicating that the fraction of lipid molecules in the gel state is increasing. Below 25°C there is gradual increase in M_1 and M_2 due to a decrease in the motional averaging of the quadrupolar interaction.

An informative way of characterising the distribution of order parameters is the relative mean square deviation of the distribution of order parameters or quadrupolar splittings, the Δ_2 parameter [3,4,25]:

$$\Delta_2 = \frac{\langle S^2_{C^2H} \rangle - \langle S_{C^2H} \rangle^2}{\langle S_{C^2H} \rangle^2} = \frac{M_2}{1.35 M_1^2} - 1$$

This parameter is very sensitive to sample inhomogeneities such as the coexistence of phases. The temperature dependence of Δ_2 for the C-14 position, shown in Fig. 6, shows a dramatic increase upon entering the transition region, and reaches a maximum at approx. 42°C which is near the centre of the calorimetric phase transition (39°C). The asymmetry in the variation of Δ_2 with temperature appears to be related to the asymmetry of the phase transition since it is sensitive to the relative amounts of the coexistent phases which are shown (see below) to exhibit an asymmetric temperature dependence.

There have been few attempts to quantitate the phase transition in terms of the fractional phase composition [6,10,24]. In one study [6], [14-

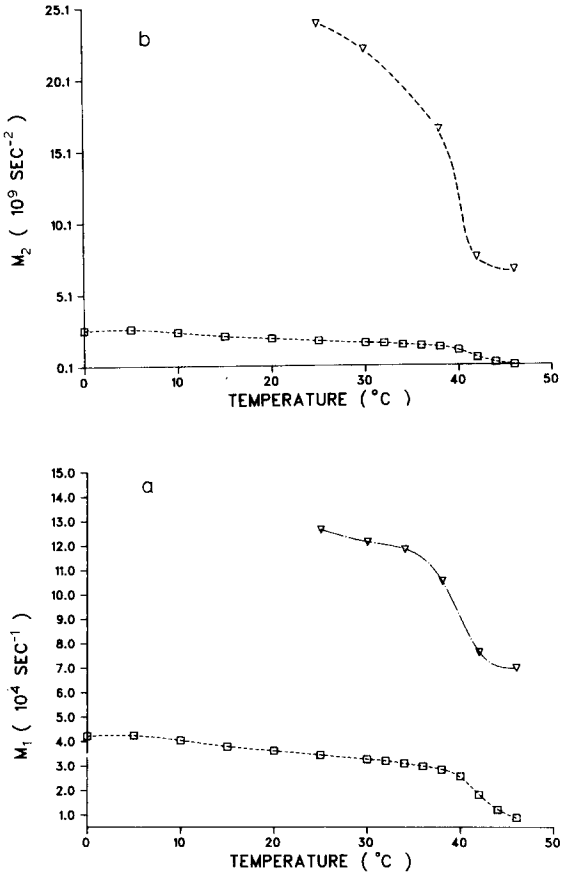

Fig. 5. Temperature dependence of (a) the first spectral moment and (b) the second spectral moment for membranes containing (□) [14-$^2H_3$] 14:0 and (▽) [4-$^2H_2$] 14:0.

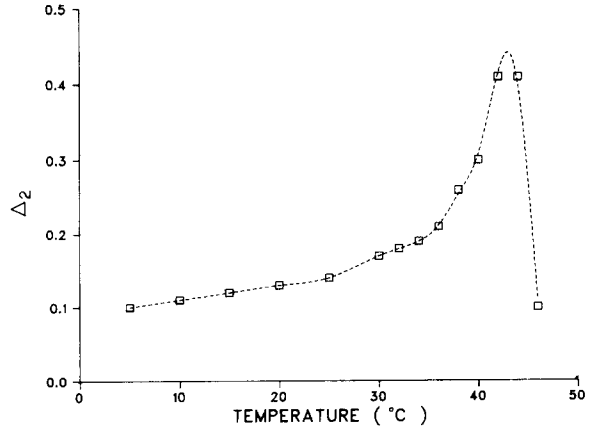

Fig. 6. Temperature dependence of the Δ_2 parameter for membranes containing [14-$^2H_3$] 14:0.

$^2H_3$]myristic acid was used to estimate the relative fractions of gel and liquid crystalline phases at several temperatures for *A. laidlawii* membranes. Since the phase behaviour of the terminal position is not necessarily representative of the entire acyl chain, we have calculated and compared the fractions of the constituent phases of membranes homogeneous in [14-$^2H_3$]- and [4-$^2H_2$]myristic acid at various temperatures. Although phase fractions can be estimated from spectral areas [10] and by theoretical spectral simulations [6], spectral moments can be used to calculate fractions more accurately in two phase systems according to [24]:

$$M_n = fM_n^L + (1-f)M_n^G$$

where M_n, M_n^L and M_n^G are the nth moments of the observed, liquid crystalline, and gel state spectra, respectively, and f is the fraction of liquid crystalline phase. The accuracy of the calculated values of f may be assessed by adding the gel state and liquid crystalline spectra (normalized) according to the calculated fractions * as described previously [24]; the composite spectra agreed with the experimental spectra to within $\pm 10\%$ (determined as described in Ref. 24) for liquid crystalline phase fractions of ≤ 0.5.

The results for membranes containing the two labelled myristic acids are shown in Fig. 7. It is clear that the transition is asymmetric for both regions of the acyl chains, suggesting that the entire chain behaves similarly. From Fig. 7, T_c values of 41 and 39°C can be estimated, which are comparable to the value of approx. 40.5 and 39.5°C determined by DSC for membranes containing [14-$^2H_3$]- and [4-$^2H_2$]myristic acid, respectively. Correcting for differences in T_c, the results indicate that the extreme regions of the acyl chains undergo a phase transition of similar width at essentially the same rate, within experimental error. The differences between the absolute temperature intervals over which the phase transition occurs, as monitored by the two labels, may be due

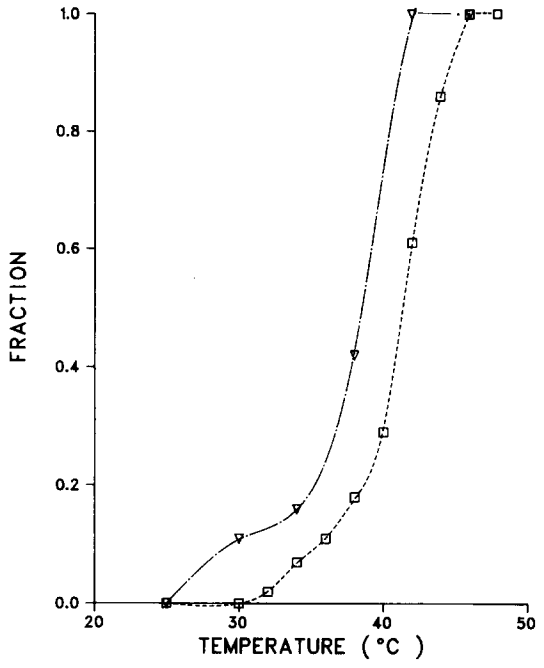

Fig. 7. Temperature dependence of the fraction of membrane lipid in the liquid crystalline phase as determined for membranes containing (▽) [4-$^2H_2$] 14:0 and (□) [14-$^2H_3$] 14:0.

to differences between the composition of the two membrane samples (see Table I) rather than an intrinsic difference between the two positions on the acyl chain. It is interesting to note that at the growth temperature (37°C) only approx. 15–35% of the membrane is in the liquid crystalline phase, as reported earlier for membranes enriched in palmitic acid [2].

30°C growth of cells

The composition of the acyl chains and the relative amounts of the various lipid headgroups in *A. laidlawii* A have been shown to vary with growth temperature [15,16]; this has been discussed in terms of lipid packing properties and phase behaviour of the individual lipid headgroup classes [16–18]. *A. laidlawii* grown in the presence of avidin lacks the ability to adjust the fatty acyl composition in response to growth temperature changes and must therefore maintain cell viability by varying the relative proportion of headgroup classes [14–16] or possibly by manipulating the carotenoid content of its membrane [36].

* To ensure that spectra were relaxed with respect to T_1, the T_1 values were measured using the inversion recovery technique [26] on a perdeuterated myristic acid-containing membrane sample and were found to agree to within $\pm 10\%$ with those reported for dipalmitoylphosphatidylcholine in the gel and liquid crystalline phases [26].

A. laidlawii B grew poorly (Table I) on a medium containing avidin and supplemented with perdeuterated myristic acid at 30°C, 7 degrees Celsius below the usual growth temperature [37]. The temperature dependence of the ^{2}H-NMR spectra of the derived membranes is shown in Fig. 8, while the variation of Δ_2 and the liquid crystalline fraction are given in Fig. 9. The phase transition occurs between 26–43°C. Unlike the study of membranes derived from cells grown at 37°C (see above), all positions of the lipid fatty acyl chain are being monitored simultaneously so that differences in their behaviour may be meaningful. Careful inspection of Fig. 8 reveals that there are no major differences between chain positions with regard to the onset and completion of the gel to liquid crystalline phase transition. The midpoint of the transition, obtained from Fig. 9, occurs at 37°C. Again, Δ_2 indicates that during the transition the sample is heterogeneous, with Δ_2 going through a maximum when the two phases are of almost equal populations. At 21°C a typical gel state spectrum is observed. As the temperature is decreased the intensity at approx. 63 kHz increases until at 15°C a large fraction of the acyl chains are reorienting slowly relative to the quadrupolar interaction. It is interesting to note that for membranes of *A. laidlawii* containing perdeuterated palmitic acid (approx. 65%), Δ_2 shows a local minimum between 20 and 30°C, below which Δ_2 increases until 0°C [3]. The results were interpreted as indicating that between 20 and 30°C the membranes are in a relatively well defined gel phase (the distribution of order parameters is not changing significantly). In the case of myristic acid-containing membranes, Δ_2 exhibits the local minimum over approx. 4 degrees (Fig. 9), and increases sharply below 23°C, suggesting that the more homogeneous membranes are able to organize into a crystalline lattice more readily than their less homogeneous counterparts containing palmitic acid. The latter result suggests that fatty acid heterogeneity may determine the organization of gel-state lipid to a greater extent than the presence of protein.

At the growth temperature (30°C) approx. 10% of the lipids are in the fluid phase. In the case of *A. laidlawii* there does not seem to be a requirement for a large fraction of the lipids to be in a fluid phase for viability. A comparison of data for the 37 and 30°C growth at the same reduced temperature ($T_c - T$) indicates that the phase compositions of the membranes are very similar. This is particularly interesting since data from the perdeuterated system gives the average behaviour of the entire chain which does not appear to differ significantly from that of the ends.

A down shift in growth temperature requires that the organism adjust its membrane composition to maintain at least 10% of its lipids in the fluid state. Most likely this requirement is associated with some membrane function. It is intriguing to note that phosphatidylglycerol is present in *A. laidlawii* membranes to the extent of 20–30% of the total lipids and that 30–35% of the phosphatidylglycerol (approx. 6–10% of the total lipid) has been reported to be in the close contact with protein [37,38].

The order-position profile may be obtained directly from spectra of membranes containing perdeuterated fatty acids by calculating oriented spectra [25,33]. The spectrum (Fig. 8e) for *A. laidlawii* containing perdeuterated myristic acid was 'de-Paked' to give the 90°-oriented spectrum, as shown in Fig. 8f. Resolved peaks corresponding to the methyl and several methylene groups are observed. Due to the complexity of this system the resolution of the various chain methylene groups is not as complete as has been reported for other systems [25,33]. However, Gaussian lines may be fitted to the 'de-Paked' spectrum as described elsewhere (Rance, M., Smith, I.C.P. and Jarrell, H.C., in preparation) to give an approximate profile of quadrupolar splitting with carbon position (Fig. 10); the data plotted are for resonances which have spectral intensity attributable to two methylene groups. Data for dimyristoylphosphatidylcholine labelled only in the *sn*-2 chain at 25 ± 2°C [28] are plotted in Fig. 10 for comparison, as well as data for the 2, 4 and 14 positions of *A. laidlawii* B membranes containing myristic acid (see above). The profile associated with the membranes agrees well with that of the model membrane system. The close agreement between the orientational order at all positions of the acyl chain of the model and the natural membrane again reflects the absence of a significant effect of protein on lipid ordering in *A. laidlawii* membranes.

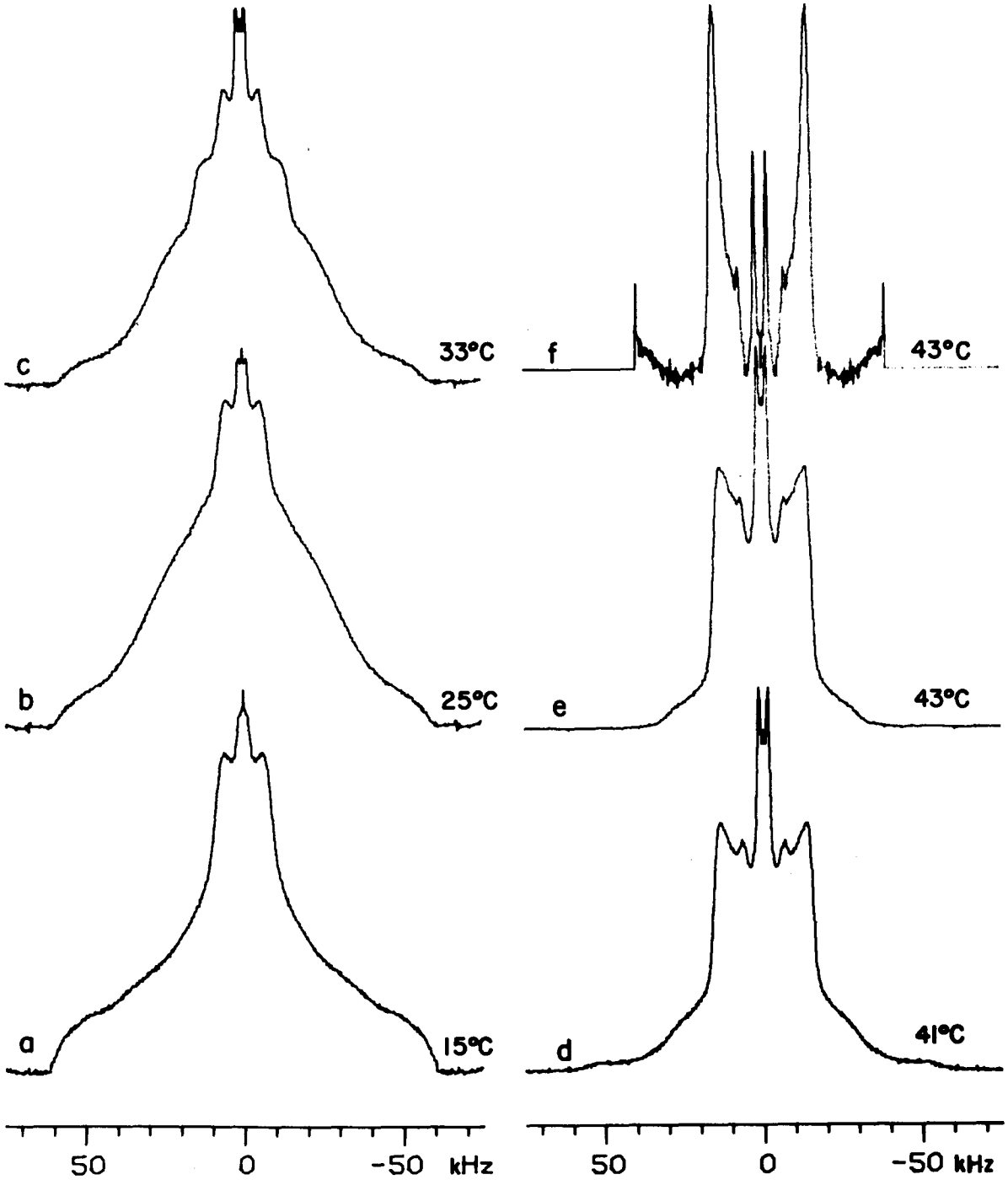

Fig. 8. (a)–(e) Temperature dependence of the ^{2}H-NMR spectra of membranes from *A. laidlawii* B grown at 30°C and containing perdeuteromyristic acid. Spectra acquired with 4.5 μs (90°) pulses, 50 μs pulse separation, 500 kHz spectral width, 0.25 s recycle time and 15000 accumulations. (f) 'de-Paked' spectrum of (e).

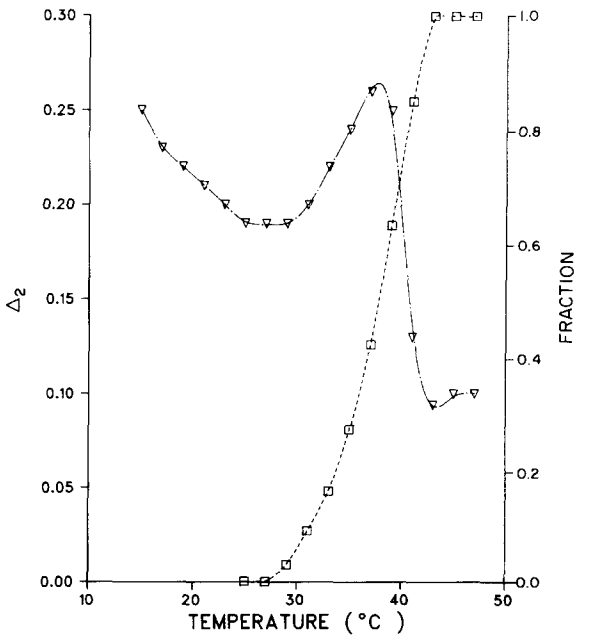

Fig. 9. Temperature dependences of (▽) the Δ_2 parameter and (□) fraction of membrane lipid in the liquid crystalline phase, as determined for membranes containing perdeuteromyristic acid.

The assignment of the quadrupolar splittings obtained for the membranes containing perdeuterated myristic acid was somewhat arbitrary. Support for the order-position profile derived using the assignments can be obtained from a moment analysis of the spectrum of membranes at 43°C.

It has been shown that the variation of quadrupolar splitting with acyl chain position can be obtained from a spectrum of membranes containing a perdeuterated fatty acid [3,39] using the empirical function

$$\nu_Q(x) = \nu_Q(0)\{1 - [(\nu_Q(0) - \nu_Q(1))/\nu_Q(0)]x^\mu\}$$

where $\nu_Q(x)$ is the quadrupolar splitting at position x. The values $\nu_Q(0)$, $\nu_Q(1)$ and μ are obtained by a least-squares fit of calculated moments to the experimental moments, as described elsewhere [3]. The form of the order position profile is assumed to be a smooth curve, as is observed for dipalmitoylphosphatidylcholine [26,27] and *A. laidlawii* membranes containing palmitic acid [1,3], and not to contain any discontinuities such as are observed for oleic acid-containing systems [4,8]. At 43°C (Fig. 8) the spectrum has the square shape and sharp cut-off in intensity which are characteristic of the 'plateau' in the variation of quadrupolar splitting with chain position found in a variety of model [26] and biological [1,3,9] systems.

An order-position profile was calculated from the moments of the 43°C spectra of membranes containing perdeuterated myristic acid and is shown in Fig. 10. The overall shape of this calculated profile is similar to that determined from the oriented spectrum as well as to that of dimyristoylphosphatidylcholine. The discrepancy in absolute values between the calculated profile and that determined directly with specific labels was also observed for the case of membranes containing palmitic acid [3]. In both cases the spectra were obtained under conditions where resonances associated with the methyl end of the acyl chain may not have been completely relaxed with respect to T_1 and this is reflected in the total spectral

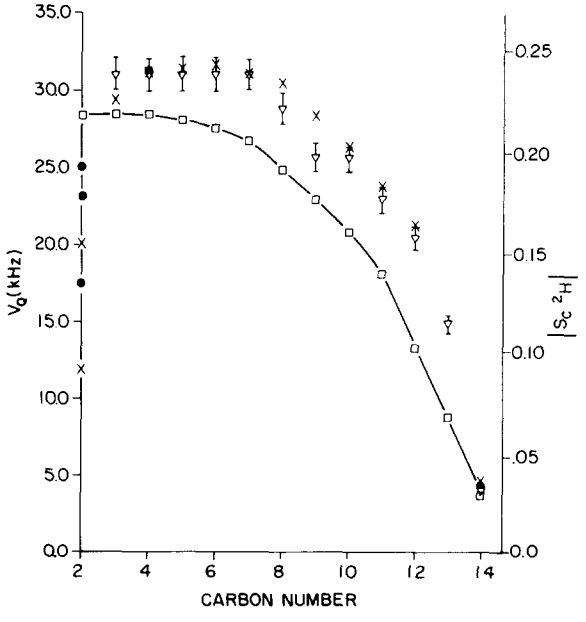

Fig. 10. Quadrupolar splitting (ν_Q) or order parameter (S_{C^2H}) vs. carbon position for *A. laidlawii* (43°C), grown at 30°C, containing perdeuteromyristic acid: □, profile calculated from spectral moments; ×, data reported for dimyristoylphosphatidylcholine (28) at approx. 25°C; ▽, profile determined from the calculated 90°-oriented spectrum (43°C); (●), data obtained for *A. laidlawii*, grown at 37°C, containing specifically labelled myristic acid (phase transition just completed).

moments and the calculated order-position profile.

Inspection of Fig. 10 reveals that membranes derived from cells grown at 37°C and 30°C have essentially the same quadrupolar splittings at C-4 and C-14 in spite of the fact that the headgroup composition of the two membranes are considerably different (Table I).

Conclusions

Isolated and intact cellular membranes exhibit very similar ^{2}H-NMR spectra for the methyl position of the acyl chains. Conclusions about the organization of isolated membranes may therefore be extended to intact cells. The lipids of fatty acid-homogeneous membranes have a considerably narrower temperature range for the gel liquid crystal phase transition than do the lipids of cells grown without avidin. The gel state lipid appears to organize into a crystalline lattice more readily than do lipids of less homogeneous membranes, indicating that chain length variations may affect the gel state organization more than does protein. All positions of the fatty acyl chains of the lipid undergo the gel to liquid crystal phase transition at essentially the same rate and over the same temperature intervals. Protein does not significantly alter the range of conformations available to the membrane lipids as evidenced by the similarity between order-position profiles for *A. laidlawii* membrane lipids and extracted lipids, and also pure phospholipid model membranes. Protein appears to increase, by approx. 20%, the linewidth associated with the spectrum of the terminal position of the lipid acyl chain as compared to that of the extracted lipid. Such an effect has been reported for protein in model [30] and recombinant [33,34] membrane studies and is under further investigation in *A. laidlawii* membranes.

Acknowledgements

The authors would like to thank Mr. D.W. Griffith and Mrs. Adèle Martin for their assistance with some of the *A. laidlawii* growths. The authors are grateful to Dr. J.H. Davis (University of Guelph) for providing the computer program for calculating 'de-Paked' spectra and to Dr. M. Rance for his assistance with calculating oriented and simulated spectra.

References

1 Stockton, G.W., Johnson, K.G., Butler, K.W., Tulloch, A.P., Boulanger, Y., Smith, I.C.P., Davis, J.H. and Bloom, M. (1977) Nature 269, 267–268
2 Smith, I.C.P., Butler, K.W., Tulloch, A.P., Davis, J.H. and Bloom, M. (1979) FEBS Lett. 100, 57–61
3 Davis, J.H., Bloom, M., Butler, K.W. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 597, 477–491
4 Rance, M., Jeffrey, K.R., Tulloch, A.P., Butler, K.W. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 600, 245–262
5 Wieslander, Å., Ulmius, J., Lindblom, G. and Fontell, K. (1978) Biochim. Biophys. Acta 512, 241–253
6 Kang, S.Y., Kinsey, R.A., Rajan, S., Gutowsky, H.S., Gabridge, M.G. and Oldfield, E. (1981) J. Biol. Chem. 256, 1155–1159
7 Kang, S.Y., Gutowsky, H.S. and Oldfield, E. (1979) Biochemistry 18, 3268–3271
8 Gally, H.U., Pluschke, G., Overath, P. and Seelig, J. (1979) Biochemistry 18, 5605–5610
9 Davis, J.H., Nichol, C.P., Weeks, G. and Bloom, M. (1979) Biochemistry 18, 2103–2112
10 Nichol, C.P., Davis, J.H., Weeks, G. and Bloom, M. (1980) Biochemistry 19, 451–457
11 Silvius, J.R., Read, B.D. and McElhaney, R.N. (1978) Science 199, 902–904
12 Read, B.D. and McElhaney, R.N. (1975) J. Bacteriol. 123, 47–55
13 McElhaney, R.N. (1974) J. Mol. Biol. 84, 145–147
14 Silvius, J.R., Mak, N. and McElhaney, R.N. (1980) Biochim. Biophys. Acta 597, 199–215
15 Christiansson, A. and Wieslander, Å. (1978) Eur. J. Biochem. 85, 65–76
16 Wieslander, Å., Christiansson, A., Rilfors, L. and Lindblom, G. (1980) Biochemistry 19, 3650–3655
17 Wieslander, Å., Rilfors, L., Johansson, L.B.-Å. and Lindblom, G. (1981) Biochemistry 20, 730–735
18 Khan, A., Rilfors, L., Wieslander, Å. and Lindblom, G. (1981) Eur. J. Biochem. 116, 215–220
19 Tulloch, A.P. (1977) Lipids 12, 92–98
20 Spencer, F. and Mangold, H.K. (1973) Chem. Phys. Lipids 11, 215–218
21 Stockton, G.W., Johnson, K.G., Butler, K.W., Polnaszek, C.F., Cyr, R. and Smith, I.C.P. (1975) Biochim. Biophys. Acta 401, 535–539
22 Wells, M.A. and Dittmer, J.C. (1963) Biochemistry 2, 1259–1263
23 Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Chem. Phys. Lett. 42, 390–394
24 Jarrell, H.C., Byrd, R.A. and Smith, I.C.P. (1981) Biophys. J. 34, 451–463
25 Bloom, M., Davis, J.H. and MacKay, A.L. (1981) Chem. Phys. Lett. 80, 198–202
26 Davis, J.H. (1979) Biophys. J. 27, 339–358
27 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4845

28 Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978) Biochemistry 17, 2727–2740
29 Engel, A.K. and Cowburn, D. (1981) FEBS Lett. 126, 169–171
30 Rice, D. and Oldfield, E. (1979) Biochemistry 18, 3272–3279
31 Seelig, J. and Browning, J.L. (1978) FEBS Lett. 92, 41–44
32 Paddy, M.R., Dahlquist, F.W., Davis, J.H. and Bloom, M. (1981) Biochemistry 20, 3153–3162
33 Bienvenue, A., Bloom, M., Davis, J.H. and Devaux, P. (1982) J. Biol. Chem. 257, 3032–3038
34 Seelig, J., Tamm, L., Hymel, L. and Fleischer, S. (1981) Biochemistry 20, 3922–3932
35 Post, J.F., De Ruiter, E.E.J. and Berendsen, H.J.C. (1981) FEBS Lett. 132, 257–260
36 Huang, L. and Haug, A. (1974) Biochim. Biophys. Acta 352, 361–370
37 Bevers, E.M., Leblanc, G., Le Grimellec, C., Op den Kamp, J.A.F. and Van Deenen, L.L.M. (1978) FEBS Lett. 87, 49–51
38 Bevers, E.M., Singal, S.A., Op den Kamp, J.A.F. and Van Deenen, L.L.M. (1977) Biochemistry 16, 1290–1295
39 Bloom, M., Davis, J.H. and Dahlquist, F.W. (1979) in Proceedings of the 20th Ampere Congress, Tallinn, Estonia (Hundla, E., Lippmaa, E. and Saluvere, T., eds.), Springer-verlag, Berlin