THE PROPERTIES OF GEL STATE LIPID IN MEMBRANES OF *ACHOLEPLASMA LAIDLAWII* AS OBSERVED BY ^{2}H NMR

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1. Introduction

Deuterium nuclear magnetic resonance (^{2}H NMR) has proven to be an informative, perturbation-free monitor of the molecular organization of lipids in model [1,2] and biological [3,4] membranes. However, in most cases studies have been limited to the liquid crystalline (fluid) state of the membrane lipids due to the difficulty of observing the broad spectrum of the gel phase. With the innovation of the quadrupole echo technique [5], and attention to various aspects of spectrometer design, it is now possible to observe these broad ^{2}H powder spectra [4,6]. We describe here a detailed study of the palmitoyl chains in the lipids of *A. laidlawii* membranes over the temperature range 1–45°C. We have shown that the gel and liquid crystalline states of the lipid coexist in slow exchange over the range of the calorimetrically-observed gel–liquid crystal transition. Only ~25% of the palmitoyl chains are still in the liquid crystalline state at the low temperature end of the calorimetrically-determined transition region. At this temperature, the orientations of those chains in the gel phase still undergo considerable motion. There is a distribution of orientational order parameters, the average being ~50% that of completely immobilized chains. This average value is that expected for chains in the all-*trans* conformation rotating about their long axes. As the temperature is lowered below the transition region, the number of immobilized acyl chains increases steadily down to 1°C where almost all the chains are immobile on the time scale of $\sim 10^{-4}$–10^{-5} s characteristic of the ^{2}H NMR spectroscopic technique.

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2. Materials and methods

Acholeplasma laidlawii B was grown at 37°C in an initially fatty acid-free tryptose broth supplemented with 13,13-d_2-palmitic acid. Cells were harvested in late log phase, osmotically lysed in distilled water, and freeze-dried for storage at −15°C. All other details of microbial growth and membrane preparation are in [7]. The acyl chain distribution in the membrane lipids, determined by gas chromatography [8], was: 16:0, 74.6%; 14:0, 13.3%; 12:0, 7.8%; unidentified 4.3%. For NMR analysis equal weights of freeze-dried membranes and distilled water were mixed and agitated gently for several hours to achieve homogeneity.

^{2}H NMR spectra were obtained using the quadrupole echo technique [5] on a Bruker SXP 4-100 spectrometer operating at 34.44 MHz. The moments of the ^{2}H spectra were calculated directly in the BNC-12 computer [9]. Sample temperature was controlled in an oven where the temperature gradient across the sample was estimated to be <0.25°C and the temperature calibration was accurate to ±0.5°C.

3. Results and discussion

The temperature dependence of the ^{2}H NMR spectra of the 13,13-d_2-palmitoyl chains in the

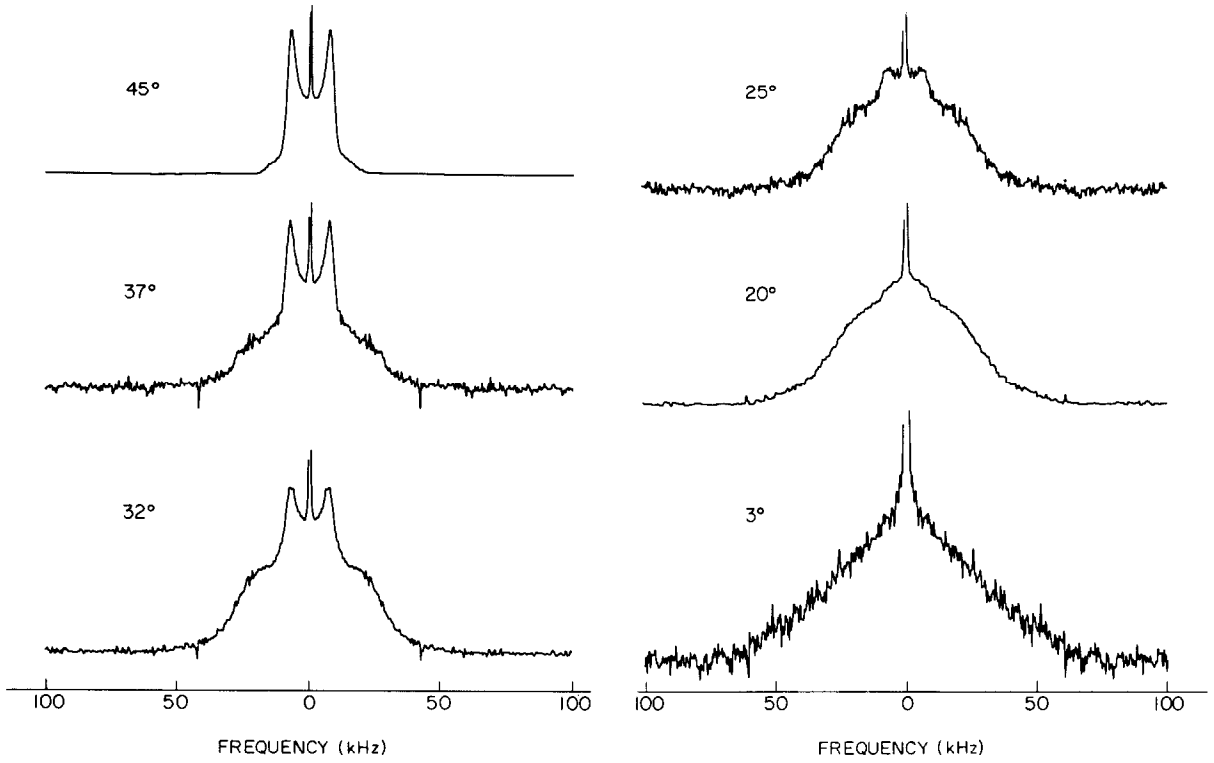

Fig.1. ^{2}H NMR spectra of the 13,13-d_2-palmitoyl chains of the lipids in membranes of *Acholeplasma laidlawii*, at the temperatures indicated, obtained via the quadrupole echo technique ($90^\circ_x - \tau - 90^\circ_y$ echo). The 90° pulse duration was 9 μs, and τ was 60 μs. Data were obtained on resonance and the resulting half spectra were reflected about the center frequency to produce those shown. The recycle time was 0.25 s.

A. laidlawii membranes is shown in fig.1. The narrow resonance in the center of the spectrum is due to ^{2}H at natural abundance in the water. The spectrum at 45°C is typical of those observed [1–4] for liquid crystalline membranes, with the advantage of extremely good signal-to-noise ratio and consequent definition of the shoulders at twice the quadrupole splitting.

The spectrum at 45°C is an almost perfect powder pattern for a single quadrupole splitting, i.e., it appears that all of the phospholipid molecules are equivalent at these high temperatures. Furthermore, measurements of the quadrupole splitting as a function of chain position at this temperature [4] have been shown to be remarkably close to those obtained in model membranes. There are two implications of these results.

1. Phospholipid molecules exchange rapidly between sites giving rise to different degrees of local orientational order, such as positions on the boundaries of proteins and those relatively far from proteins.
2. The average perturbation of the local orientational order of the acyl chains of phospholipid molecules by the proteins must be relatively small at 45°C.

The second implication suggests that the immobilization of boundary lipids containing ESR spin labels [10] is a phenomenon not characteristic of natural phospholipids in biological membranes in the liquid crystalline phase.

On decreasing the temperature to 37°C, within the range of the broad liquid crystal–gel phase transition (43–27°C) observed calorimetrically [11], a broader spectrum with an average splitting of ~60 kHz appears beneath that due to the liquid crystalline phase. The

presence of two distinguishable resonances demonstrates the coexistence of the two states of the membrane lipid with an interconversion rate less than the difference between the two quadrupole splittings ($<10^4$ s^{-1}). Thus, rather than maintaining a state whose properties are intermediate between those of the liquid crystalline and gel states, discrete populations of the two states are present. The coexistence of two states of the membrane lipid in *A. laidlawii* has also been detected recently by ESR studies of spin-labelled fatty acids [8]. Similar results have been obtained by ^2H NMR for perdeuterated dipalmitoyl phosphatidylcholine at temperatures within the range of the much narrower enthalpic transition of the chains [6]. The coexistence of two thermodynamic states in model membranes containing lipids with different transition temperatures has been inferred from a wide variety of physical measurements. However, the ^2H NMR measurements represent the most direct observation of this coexistence and provide a reliable method of measuring the partition of the phospholipid molecules between the two regions.

On decreasing the temperature further into the range of the thermal transition, the spectra in fig.1 indicate increasing amounts of the broad component of the spectrum at the expense of the liquid crystalline component. By 25°C, the broad component, with a quadrupole splitting of ~60 kHz, dominates the spectrum. This value of the splitting is that expected for a high degree of molecular order but a rapid rate of rotation about the long axis of the molecules.

Calorimetric studies indicate that at <27°C the liquid crystal to gel transition of the palmitoyl chains is essentially complete [11]. However, decreasing the temperature to 3°C results in a continuous increase in the width of the ^2H spectrum (fig.1) which has intensity out to ±60 kHz from the center. At 3°C the width is ~110 kHz. The lineshape of the 3°C spectrum is peculiar, suggestive of insufficient radiofrequency power to observe the outer components at their true intensities. Decreasing the pulse angle from 90° (9.0 μs) to 30° (3.0 μs) yields a more uniform distribution of power across the spectrum with retention of a satisfactory echo. This can only be done, however, with a considerable loss in signal-to-noise ratio. The spectrum obtained at 1°C using this approach is shown in fig.2. The large intensity near ±60 kHz is indicative of almost total cessation of motional

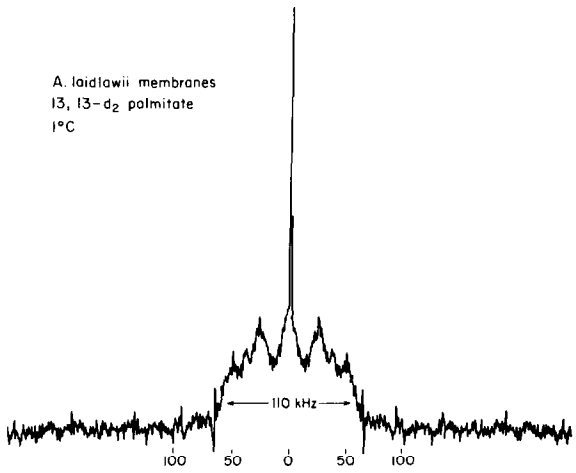

Fig.2. The ^2H NMR spectrum of the sample used for fig.1, at 1°C, employing 30° pulses of duration 3.0 μs, a τ value of 60 μs, and a recycle time of 0.25 s.

averaging of the quadrupolar interaction for a large fraction of the hydrocarbon chains. For a quadrupole coupling constant of 167 kHz, a band width of 126 kHz is expected in the absence of motion. Thus, on decreasing the temperature from 25–0°C the rapid rotation of the fatty acyl chains is progressively frozen out.

Further insight into the motional behaviour responsible for the spectra in fig.1,2 is gained from measurement of their second moments (M_2). An increase in the M_2 is indicative of decreased efficiency of motional averaging of magnetic tensor components [12]. The M_2 of the ^2H NMR spectra of the *A. laidlawii* membranes are shown as a function of temperature in fig.3. The steep increase in M_2 from 45–25°C is due to the increase in the fraction of the phospholipid molecules of the system in the gel state. The value of the M_2 at 25°C is, in fact, fairly close to that expected on the basis of the picture of the gel state in model membranes provided by X-ray diffraction studies [13,14]. The X-ray data indicate that the molecules are essentially in a fully extended (all-*trans* conformation) state and have 'rotational disorder', i.e., the charge distribution of the molecules, as measured by X-ray, has approximately cylindrical symmetry about their long axes. This state of 'rotational disorder' persists over a wide temperature range in model membranes and is usually pictured as being

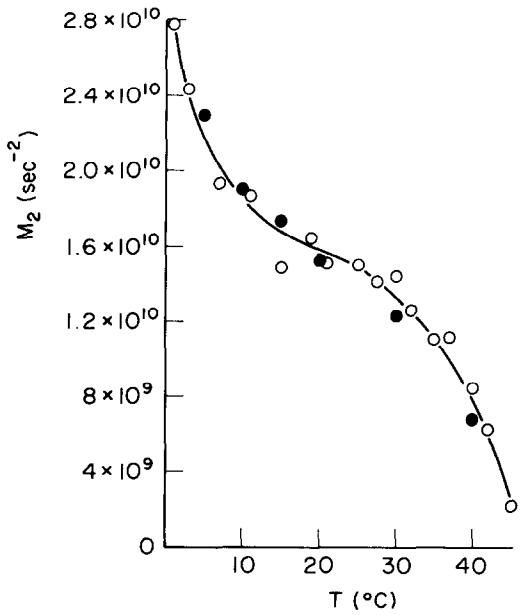

Fig.3. Temperature dependence of the second moments of the ^{2}H NMR spectra of *Acholeplasma laidlawii* membranes enriched in 13,13-d_2-palmitic acid.

associated with rapid rotation of the molecules about their long axes.

Studies of perdeuterated dipalmitoyl phosphatidylcholine model membranes [6] have shown that although the average orientational order in the gel state just below the liquid crystal–gel transition temperature is consistent with this simple interpretation of the X-ray diffraction data, the hydrocarbon chains of a progressively larger number of phospholipid molecules become completely immobilized as the temperature is decreased below the transition temperature. The increase in the M_2 at <20°C in fig.3 shows that the same type of behaviour is found in *A. laidlawii* membranes below the liquid crystal–gel transition region.

We wish to emphasize, however, that at the present time the variation of M_2 at <20°C has only qualitative significance since corrections due to the finite length of the radiofrequency pulses will enhance the intensity of the NMR signals in the wings of the spectrum relative to that near the center (see fig.1,2) thus giving rise to a systematic increase in the M_2 relative to those plotted in fig.3. A precise formula for the correction to the NMR spectrum due to finite pulse length has been derived [15]. We intend to present a more precise analysis of the temperature dependence of the moments of the ^{2}H NMR spectrum of *A. laidlawii* membranes in a subsequent publication, which makes use of this correction.

4. Conclusion

By means of improved ^{2}H NMR techniques we have been able to obtain a clear picture of the orientational ordering and motional behaviour of the membrane lipid acyl chains in *A. laidlawii* at temperatures above and below the onset of the liquid crystal–gel phase transition. During the enthalpic transition the ^{2}H NMR spectra of both states of the lipid are observed. This implies that individual molecules exchange between these states at $\lesssim 10^4$ s^{-1}. At temperatures just below this transition, as a first approximation, the ^{2}H NMR spectra imply that the only rapid motion remaining is rotation about the long molecular axes. Further decreases in temperature lead to cessation of this motion and at 1°C the lipid hydrocarbon chains are essentially immobile.

As well as providing detailed insight into the membrane behaviour of this organism, the methods employed here should allow a variety of previously difficult studies such as the observation of lipid immobilized by protein or sterols. They also yield an extraordinary decrease in the time required to observe liquid-crystalline lipids, and hence permit the use of smaller quantities of material and the observation of systems with low lipid content.

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