LIPID CHAIN ORDER IN *ACHOLEPLASMA LAIDLAWII* MEMBRANES

What does ^{2}H NMR tell us?

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1. Introduction

Deuterium magnetic resonance (DMR) has provided much information about the states of lipid hydrocarbon chains in bilayers [1,2] or in *A. laidlawii* membranes [3] and it is with the interpretation of the latter results, and their comparison with the results of Raman scattering, that we are concerned. The Raman spectrum of pure phosphatidylcholines shows 3 bands in the region 1060–1130 cm^{-1} which have been associated with motions of the hydrocarbon chains. In particular, the temperature-dependence of the '1130 cm^{-1}' band has been determined for dipalmitoyl- and distearoylphosphatidylcholine (DPPC and DSPC) [4–8]. It has been generally accepted that the reduction of the intensity of the '1130 cm^{-1}' band as the temperature increases towards T_c, the gel to fluid phase transition temperature, is due to the appearance of *gauche* bonds in a chain. This has been quantified in [8] where good agreement between experiment and theory was obtained. This confirmed the view that there are a non-negligible number of *gauche* bonds excited as low as −50°C, and this number ($< n_g >$) was calculated to increase to 2–4/molecule just below T_c. When the bilayer melts $< n_g > \approx 7$, 10 and 13.5 for DMPC, DPPC and DSPC, respectively.

The measurements in [3] on *A. laidlawii* membranes have shown a well-defined line for the 13 CD_2 position of DPPC with a splitting $\Delta\nu_Q \approx 20$ kHz at 45°C. At 37°C a line with a larger splitting ($\Delta\nu_Q \approx 50$ kHz) appears. As the first line disappears the splitting of the second line increases to ~60 kHz at 25°C and to ~110 kHz at 1°C. This has been interpreted as follows: The first ($\Delta\nu_Q \approx 20$ kHz) line arises from chain motion in a fluid-like phase while the second ($\Delta\nu_Q \simeq 50$–110 kHz) is associated with chain motion in a gel-like phase. The interpretation continues that the 60 kHz splitting can be identified with a high degree of molecular order but with a rapid rate of rotation about the long molecular axis, and that the increase of the splitting to 110 kHz at 1°C indicates an almost total cessation of rotational motional averaging of the quadrupolar interaction, so that the rapid rotation of the fatty acyl chain is frozen out. Thus, for temperatures a few degrees below T_c the only significant rapid motion is rotation about the long molecular axis and at 1°C this has been essentially frozen out. Note that this says that there are essentially no *gauche* bonds on a chain because, if there were then in the absence of rapid transitions between states of a chain, many splittings would be observed. If this is so then there is a significant disagreement between the results of DMR and Raman spectroscopy because, in general, lipid chains might be more disordered in the gel phase of the *A. laidlawii* membrane than a pure DPPC bilayer. Here we will attempt to resolve this disagreement.

2. Model of lipid chains in *A. laidlawii*

Our intention is to construct a simple model of the *A. laidlawii* membrane, which is in accord with what is known, and then to study the probabilities of finding a chain to be in various states of interest, notably the ground (all-*trans*) state, or any of the single-kink states. A model for lipid hydrocarbon chain dynamics in lipid bilayers and the effects of intrinsic molecules (e.g., cholesterol and proteins) has been presented in [9]. This model has also been used

[10] to understand in a simple way the dependence of T_c upon chain length, the enhancement at T_c of the diffusion of Na^+ out of DPPC vesicles, the DMPC–DPPC phase diagram in the region of 300 K, and to analyze the DMR results in [11]. This model, together with a theory of Raman scattering by transverse optic hydrocarbon chain modes has successfully accounted for the temperature-dependence of the '1130 cm^{-1}' band [8]. As adapted to this case, the model assumes that each site of a triangular lattice can be occupied by a lipid chain, while a set of 91 such sites in the shape of a hexagon represents an average intrinsic protein of mol. wt 65 000. Each lipid chain can exist in:

(i) an all-*trans* ground state, G, which projects an area A_0 (20.4 $Å^2$) on to a plane perpendicular to the long molecular axis (here, the director) and has internal energy 0;

(ii) An excited 'melted' state, E, of internal energy E_E, degeneracy D_E and area A_E (34 $Å^2$); or

(iii) A number of intermediate states which can be excited, without much steric hindrance, below T_c.

If (iii) is excluded the model becomes similar to that in [12] and [9]. The intermediate states have been described in [8,10]. The states (i) and (iii) are known explicitly so that order parameters can be calculated for each group in the chain. The details of the 'melted' state, E, are unknown so that its order parameters must be determined from experiment. The chains interact via a quadrupole–quadrupole interaction as in [13], and there is an effective lateral pressure acting in the hydrophobic region as introduced [14] which we take to arise from the interactions involving polar headgroups. The order parameters for either of the C–D bonds (assumed equivalent) of the k-th group is $S_k = \frac{1}{2}(3\cos^2\theta_k - 1)$, where θ_k is the angle between the *C–D* axis and the director. These can be calculated explicitly for the all-*trans* and intermediate states. We have calculated S_k ($k = 3, \ldots, 14$) when the chain is in its 'melted' state E, by fitting the thermal average of each S_k to those measured [11] at 30°C. Note that we have ignored lipid rotation or diffusion, and have assumed that there are no fluctuations in the direction of the long molecular axis. Such motions do not affect the Raman '1130 cm^{-1}' band intensity. As we have seen, all the lipids were taken as DPPC for simplicity, and the average protein was given a mol. wt 65 000. We took the concentration, c, of intrinsic protein as $c = 0.01$ and assumed that there is a phase boundary at $c = 0.05$ (82.3 wt % protein). The lipid chain dynamics were studied as in [8–10] in a mean field approximation.

3. Results

Figure 1 shows the phase diagram, with our model corresponding to $c = 0.01$, as well as the specific heat. The latter is ~14° wide in general agreement with DSC measurements [15]. Table 1 shows the calculated splittings $\Delta\nu_Q(k)$ for groups $k = 4, 8, 12$ and 16 (methyl) in a DPPC chain for different temperatures. In addition we show the probabilities of finding the chain in its all-*trans* state, P_G, or any single-kink state, P_{1k}. Note that even though $P_G = 0.634$ at 270 K, $\Delta\nu_Q(12) = 119$ kHz which agrees with that observed [3] at 1°C. At 318.2 K, $\Delta\nu_Q(12) = 23$ kHz, agreeing with the ~20 kHz splitting observed at the 13 position at 45°C. At ~270–312 K there is a phase with a splitting of >100 kHz for $\Delta\nu_Q(12)$ even though $P_G < 0.62$ and the probability of exciting a kink rises to >0.2. The measurements in [3] are thus in accord with a picture in which there are *trans–gauche* conformational changes, on a time-scale short compared to the DMR time-scale, so that the splitting is a thermal average of the contributions from the different conformers and the chain is not only in the all-*trans* state. Further, at 270 K the agreement shows

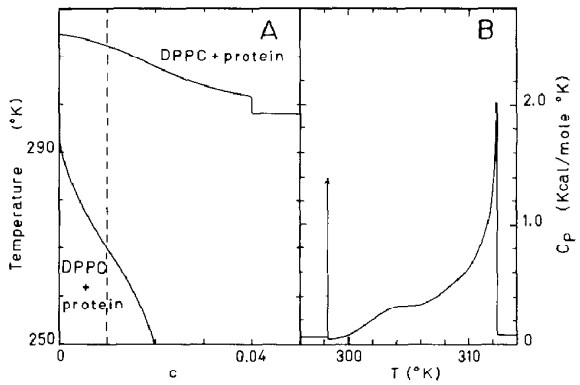

Fig.1(A). Phase diagram of a DPPC bilayer with an intrinsic protein of mol. wt 65 000. The dashed line at $c = 0.01$ indicates the protein concentration chosen for the model of the *A. laidlawii* membrane. (B) Specific heat, C_P, of the *A. laidlawii* membrane model. The line at 298.3 K represents a δ-function which contributes ~0.4 kcal/mol to the transition enthalpy. The inclusion of other lipids and proteins would smear out the two peaks.

Table 1
Calculated DMR splittings for positions 4, 8, 12 and 16 in a DPPC chain, and the probabilities of the chain being in its all-*trans* (P_G) or any single-kink (P_{1k}) state as a function of temperature

T (K)	$\Delta\nu_Q$ (k) (kHz)								Probabilities			
	4		8		12		16		P_G		P_{1k}	
270	120.3		119.3		118.7		35.1		0.634		0.169	
280	119.8	32.0	118.7	31.1	118.2	26.3	34.9	3.6	0.619	0.034	0.189	0.034
293.2	118.1	24.7	116.9	24.5	116.4	19.5	34.0	1.8	0.578	0.019	0.194	0.031
305.2	113.5	29.3	111.9	28.8	111.3	24.1	31.5	3.1	0.494	0.030	0.212	0.044
310.2	110.1	31.9	108.2	31.3	107.5	26.8	29.9	3.8	0.448	0.037	0.217	0.051
318.2	28.0		27.6		23.0		2.8		0.027		0.042	

Note the two-phase region at ~270–~312 K

that the fast motion involves only transitions between different conformers with essentially no contribution from molecular rotation. Table 1 shows that at ~270–312 K spectra from two phases will be simultaneously observed. The nearly protein-free phase contributes the large splitting while the phase with $c = 0.01–0.05$ contributes the other. The large splitting appears at ~312 K and gets stronger as T decreases while at the same time the narrow splitting line disappears. This is in general agreement with [3] although the large splitting seen in [3] is ~50 kHz at ~310 K and not 108 kHz. This is probably because the lipid molecules are undergoing additional rapid motion near 310 K such as rotational, as suggested [3], or fluctuations in the orientation of the long molecular axis. This additional motion then gets frozen out as the temperature is reduced to ~270 K. Note that such additional motion would not be reflected directly in the '1130 cm^{-1}' Raman scattering band from lipids in the *A. laidlawii* membrane since this mode involves relative CH_2 group motion and is not influenced directly by motion of the molecule as a whole. A 60 kHz splitting was observed [11] in pure DPPC which is in accord with the idea of additional rapid motion.

4. Conclusions

We have calculated $\Delta\nu_Q$ for various C–D positions along the 2 chain of DPPC in the *A. laidlawii* membrane, modelled as a DPPC bilayer containing a large (mol. wt 65 000) protein of $c = 0.01$. The results are in general agreement with those in [3], and the analysis shows that, contrary to what was suggested, there are transitions between different chain conformational states, through the excitation of *gauche* bonds, on a time-scale rapid compared to the DMR time-scale. Near 270 K there are essentially no effects associated with molecular rotation. This result is entirely in accord with that of the temperature-dependence of the '1130 cm^{-1}' Raman band intensity in pure DPPC. The observed splitting of ~60 kHz near 310 K, rather than ~108 kHz as calculated, is probably due to DPPC molecular rotation and/or fluctuations in the orientation of the long DPPC axis.

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