^{13}C NUCLEAR MAGNETIC RELAXATION TIMES AND MODELS FOR CHAIN MOTION IN LECITHIN VESICLES

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Received 19 April 1972

1. Introduction

Lecithin vesicles are widely used as model systems for the bilayer organisation of lipids in biological membranes. The permeability of such vesicles appears to be determined by the packing of the fatty acid chains and is strongly influenced by their structure [1]. In so far as it affects the molecular motion of the lipids, chain packing can be studied by ESR, using spin labels [2], and by NMR. Recently, spin lattice relaxation times (T_1) of ^{13}C nuclei at neutral abundance in lecithin vesicles have been reported [3, 4]. Spin lattice relaxation times, which are inversely related to the correlation time(s) for molecular motion, are potentially of considerable value in studying these systems, since the measurements are sensitive to molecular motion on the time scale of interest, and involve no chemical modification of the lipid structure.

In view of the complexity of the motions involved, only a qualitative interpretation of the T_1 values in terms of molecular motion has hitherto been attempted. It is clear from the pattern of T_1 values of a lecithin molecule in a bilayer that there is a decrease in the effective correlation time of each $-CH_n$-group from the glycerol moiety towards the terminal methyl groups of the fatty acid chains and of the $-\overset{+}{N}Me_3$ head-group [4]. However, it is not possible to separate intuitively the contributions of the different types of motion to the effective correlation time, nor to use the T_1 measurements to describe the motion about the carbon–carbon bonds in detail. We now report calculations of ^{13}C T_1 values predicted by a number of physically reasonable models for the motion of the lecithin hydrocarbon chains, in order to arrive at a more detailed interpretation of the observed values. Only carbons 2, 3, 14, 15 and 16 of the chains of dipalmitoyl lecithin can be clearly resolved at 25 MHz [3], so that only a limited amount of experimental data is presently available. However, we shall show that even this limited data allows us to determine the type of motion which dominates the relaxation times, to exclude some models of chain motion, and to indicate the type of model which is likely to be correct.

2. Methods

Dimyristoyl lecithin (DML) was synthesised by the method of Robles and Van den Berg [5]. Dipalmitoyl lecithin (DPL) was obtained from Koch-Light. Preparation of the sonicated lecithin vesicles and measurement of ^{13}C T_1 values have been described previously [3, 4].

The effects of internal motion in an isotropically tumbling molecule on nuclear magnetic relaxation have been described theoretically by Woessner [6] and Wallach [7], and examples in simple molecules have been described (for example, [8]). We shall assume throughout that ^{13}C T_1's are determined by C–H dipolar relaxation [3, 9].

The method used consists of calculating the angular autocorrelation function for each CH_2-group down the chain. Calculation of the correlation function for the vesicle motion is straightforward, since this motion is isotropic. The correlation function for the n^{th}-carbon is then calculated from that of the $(n-1)^{th}$-carbon as described by Wallach [7], by multiplication by a suitable rotation matrix, obtained from the

correlation time, τ_{cc}, for the motion about the n^{th}-carbon–carbon bond. τ_{cc} is defined as $1/6\, D_r$ where D_r is the rotational diffusion coefficient. The effective correlation time required for calculation of T_1 from the Solomon–Bloembergen equation [10] is the integral of this correlation function. Wallach [7] gives an analytical expression for this integral. This permits a relatively straightforward calculation of T_1 from individual correlation times when the number of bonds about which there is internal motion is relatively small. However, we have found that the computation time required for chains of more than about 7 carbons becomes prohibitively long. We have therefore carried out a numerical integration of the correlation function instead of evaluating the analytical expression for the integral. Under the conditions used here, this method gives results within 3% of those given by the analytical expression [7], and is at least a factor of 1,000 faster for long chains. We have avoided the use of the simplified approach described by Wallach [7] for the treatment of successive internal motions, as this gives accurate results only when each internal motion is 10 times faster than the preceding one [11]. The calculations will be described in more detail elsewhere, together with some general theoretical results for spin–lattice and spin–spin relaxation times of ^{13}C, ^{1}H and ^{19}F nuclei in alkyl chains [11].

The calculations have been performed for CH_2-groups throughout, and the experimental T_1 value for the terminal methyl has been multiplied by 3/2 to facilitate comparison.

3. Results and discussions

Three types of motion are considered in the calculation of the T_1 values; the isotropic tumbling of the whole vesicle, for which a correlation time of approx. 10^6 sec is calculated from the Stokes–Einstein equation. Secondly, motion of the lecithin molecule as a whole about its long axis, which appears from ESR experiments to have a correlation time of 10^8-10^9 secs [2], and finally motion about individual carbon–carbon bonds in the fatty acid chains. The observed T_1 values are in the range 0.1–5.0 sec (see figs. 1 and 2), and they increase with increasing temperature [4] indicating that for all carbons $(\omega_H+\omega_C)\tau_{eff} > 1$ (where ω_H and ω_C are the operating frequencies for protons and carbon – 6.28×10^8 and 1.57×10^8 radians/sec, respectively – and τ_{eff} is the effective correlation time for an individual carbon).

Models involving only the first two types of motion, that is where the lecithin chains behave as rigid rods rotating about their long axes, can immediately be eliminated, since they would predict identical T_1 values for all carbons in the chain. Once motion about the carbon–carbon bonds is introduced, with correlation times (τ_{cc}) short enough to account for the observed T_1 values, then the rates of vesicle tumbling and of axial motion have no significant effect on the T_1 values of carbons beyond the first, provided they are less than 10^9 sec^{-1} (see table 1). This findings means that in attempting to account for the observed T_1 values we need consider only motion about carbon–

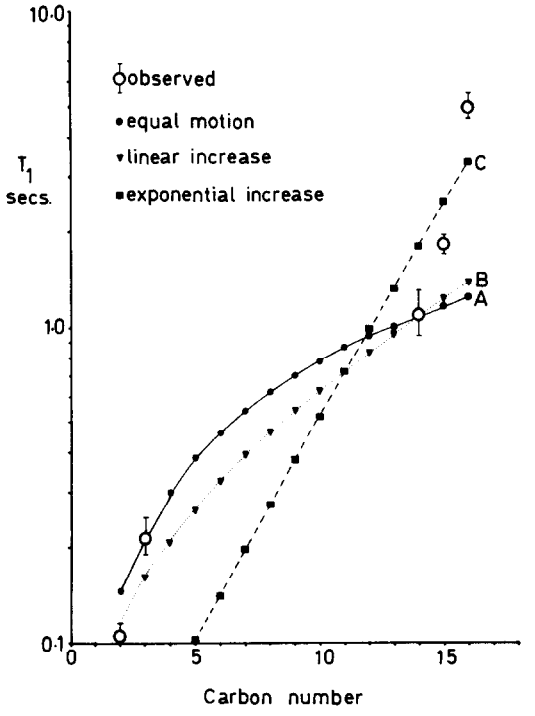

Fig. 1. Comparison of experimental ^{13}C T_1 values for dipalmitoyl lecithin vesicles with values predicted by three simple models for chain motion. The parameters used to generate the curves A, B and C are given in table 2. For curve C, the T_1 values predicted for carbons 2 and 3 are 0.034 and 0.047, respectively. If the parameters in the 'exponential increase' model are chosen, like those for curves A and B, to give the best fit to the data points for carbons 2, 3 and 14, the curve obtained is essentially superimposable on curve B.

Table 1
Effects of vesicle tumbling and of axial motion on ^{13}C T_1 values in lecithin vesicles.

Vesicle correlation time	Axial motion correlation time	T_1 (sec)*				
		1†	2	3	4	5
10^{-5}	none	20.15	0.11	0.16	0.25	0.34
10^{-6}	none	2.01	0.11	0.16	0.25	0.34
10^{-7}	none	0.20	0.11	0.16	0.25	0.34
10^{-8}	none	0.03	0.08	0.16	0.25	0.34
10^{-6}	10^{-8}	0.05	0.09	0.16	0.25	0.34
10^{-6}	10^{-9}	0.03	0.08	0.16	0.26	0.35

* For brevity, only values for the first five carbons are given.
† For this carbon, $(\omega_H+\omega_C)\tau_{eff} \geqslant 1$ under these conditions.

carbon bonds in the alkyl chains, and that any inaccuracies introduced by using the Stokes–Einstein equation to calculate the vesicle correlation time will be completely negligible. Furthermore, the results shown in table 1 indicate that the T_1 values obtained for lecithin vesicles should be directly comparable to those obtained for membrane fragments [12] despite the different size of the particles. Wallach [7] states that when an internal motion is rapid compared to the isotropic motion, the effective correlation time is simply an angular factor (~0.1 for the tetrahedral angle) multiplied by the correlation time for the isotropic motion. The present calculations indicate that when the isotropic motion is slow this is true only for the first carbon; for the subsequent carbons the isotropic motion has no effect (see [11] for further discussion of this point).

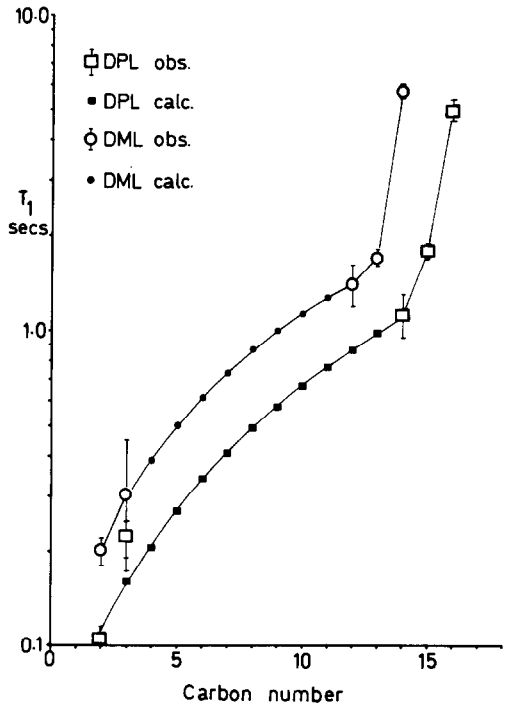

Fig. 2. Comparison of experimental ^{13}C T_1 values for dipalmitoyl and dimyristoyl lecithin vesicles with values predicted by the 'hybrid' models whose parameters are given in table 2.

The values of τ_{cc} ($\ll 10^{-10}$ sec) required to explain the observed T_1's suggest that the motion involved is oscillatory (i.e. within a conformation) rather than rotational, involving conformational change. If the motion is oscillatory, then the correlation time, τ_{cc} is the product of the true correlation time and a term describing the amplitude of the oscillatory motion [11, 13]. However, since it is impossible to distinguish in these experiments between a decrease in true correlation time and an increase in amplitude of oscillation down the chain (it is likely, of course, that both occur), we shall continue to use the τ_{cc} values alone.

Fig. 1 shows a comparison of the experimental T_1 values for the chain carbons of dipalmitoyl lecithin vesicles [4] with those calculated from some simple models of chain motion. The parameters used for each of the calculated curves, which were chosen to give the best fit to the data for each model, are given in table 2. It is clear that any model involving equal motion about all carbon–carbon bonds in the chain (curve A), which gives a linear increase in T_1 down the chain, is not compatible with the sharp increase in

Table 2
Correlation times, τ_{cc}, used to generate the curves in figs. 1 and 2*.

Fig.	Curve	Type of model	τ_{cc} values (10^{-10} sec) for bond number														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A	Equal motion	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
1	B	Linear increase	2.00	1.90	1.80	1.70	1.60	1.50	1.40	1.30	1.20	1.10	1.00	0.90	0.80	0.70	0.60	0.50
1	C	Exponential increase	10.00	7.40	5.48	4.05	3.00	2.22	1.64	1.22	0.90	0.66	0.49	0.36	0.27	0.20	0.15	0.11
2	DML	Hybrid†	1.00	0.96	0.92	0.88	0.85	0.81	0.78	0.75	0.72	0.69	0.66	0.64	0.38	0.015		
2	DPL	Hybrid†	2.00	1.86	1.73	1.61	1.50	1.39	1.29	1.20	1.12	1.04	0.97	0.90	0.84	0.78	0.15	0.023

* For all curves, the vesicle correlation time is 10^{-6} sec, and there is no axial motion.
† These models consists of a rather shallow exponential decrease in τ_{cc} for most of the chain, followed by a sharp decrease for the last two bands.

T_1 seen for carbons 14, 15 and 16. A model in which τ_{cc} decreases linearly from carbon 1 to carbon 16 (curve B) is similarity inadequate. If it is supposed that τ_{cc} decrease exponentially down the chain a somewhat better fit is obtained (curve C) but in order to reproduce the sharp increase in T_1 at C15 and C16 even approximately, it is necessary to use parameters which give T_1 values for C2 and C3 which are substantially too short.

It appears then to be necessary to postulate a model involving little or no decrease in τ_{cc} in the first part of the chain, followed by a sharp decrease toward the terminal methyl. Such a model can fit the data for both dipalmitoyl and dimyristoyl lecithin, as shown in fig. 2. Is must be emphasised that a unique fit of this model to the data is not claimed. In particular, the point along the chain at which the decrease in τ_{cc} begins cannot be defined with any accuracy. A much more accurate description of the chain motion will be possible when T_1 data becomes available for the central section of the chains from ^{13}C- and ^{19}F-labelled lecithins. However, even with the present limited data, we have demonstrated that a number of models of chain motion can be eliminated, and that a marked increase in motion in the terminal region of the chain must be postulated.

Fig. 2 clearly shows that as the hydrocarbon chains in the lecithin molecules get shorter, the motion increases right back to the beginning of the chain; the whole bilayer becomes more fluid. The changes between dipalmitoyl and dimyristoyl lecithin illustrate the sensitivity of T_1 measurements to small differences in motion. The increased fluidity of the chains in dimyristoyl lecithin shown by these measurements correlates with the lower transition temperature [14] and greater glycerol permeability [1] for dimyristoyl compared to dipalmitoyl lecithin.

4. Discussions

We have shown that the ^{13}C T_1 values of lecithin molecules in vesicles are largely unaffected by the tumbling rate of the vesicle, and that the anisotropic axial motion of the whole molecule is also insignificant as a T_1 relaxation process when it has a correlation time longer than 10^{-9} sec. This is short compared to spin label estimates of this motion. The T_1 values of

carbons in the fatty acid chains of the bilayer are determined by the summation of motion about the C–C bonds, and this is probably dominated by oscillatory motion within a given chain conformation.

Models involving an exponential decrease in τ_{cc} down the chain do not fit the experimental data. The simplest class of model with which we have been able to achieve a satisfactory fit involves little or no decrease in τ_{cc} for the major part of the chain, with a very pronounced decrease near the terminal methyl group. The same type of model can fit data from lecithins of different chain lengths, but the numerical values of τ_{cc} required are different. This model is also in qualitative agreement with the ESR "order parameter" data [2], which reflects motion on a much longer time scale.

These results indicate that when T_1 values become available for all the carbons in the fatty acid chains, calculations of the kind described here will permit a quantitative description of the motion of the chains.

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