COMPARISON OF ^{13}C SPIN-LATTICE RELAXATION TIMES IN PHOSPHOLIPID VESICLES AND MULTILAYERS

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Summary

Spin-lattice relaxation times (T_1's) for chain carbons of phospholipids in vesicle and multilayer models for biological membranes have been compared. The similarity of the results in the two systems suggest that unlike proton spin-spin relaxation times, ^{13}C T_1's depend on a chain motion which is quite independent of the size or shape of a lipid bilayer. A β-coupled isomerization of C-C bonds is suggested as a motion having this property.

The motional properties of lipids in biological membranes are important factors for the proper execution of numerous cellular functions. These properties have been studied in real and artificial membranes by several techniques including some which do not perturb native structure, namely measurement of proton or carbon-13 magnetic resonance spin relaxation times. Despite agreement over the qualitative relation of spin relaxation times to membrane fluidity (increased relaxation times imply increased fluidity), there is a divergence of opinion over the details of the motional properties which determine these parameters. [1-4]

One detailed interpretation deals with proton spin-spin relaxation times, T_2's, as measured by line width, in model membranes.[1] Line widths are found to be strongly dependent on the curvature of the lipid bilayer, decreasing by orders of magnitude in going from a multilayer structure in which the bilayer is planar to a vesicle structure in which it has a radius of curvature equal to $\sim 150\text{Å}$. The interpretation of this sensitivity is that line widths are determined by off-axis motions of hydrocarbon chains which occur more frequently in the disordered vesicle system. The implication is that for proton line width measurements the overall geometry or morphological character of the preparation may dominate effects due to intermolecular interactions among membrane constituents.

In view of this limitation in the use of proton spin-spin relaxation data, it is important to examine other means of measuring membrane fluidity in the hope of finding a method more uniquely related to molecular characteristics and interactions. Carbon-13 spin-lattice relaxation times (T_1's) have recently been used to examine motional properties of membrane preparations.[3,5] Even without detailed analysis they offer an advantage over proton measurements in that interactions leading to relaxation are better defined. In the case of a methylene carbon, they are purely dipole-dipole interactions between carbon and directly bonded protons.[3] We will present here evidence that carbon-13 relaxation times of methylenes in bilayer systems are less sensitive to morphological changes than proton spin-spin relaxation times and thus are potentially more easily linked to the motional characteristics of the molecule of interest.

The evidence is obtained by a direct comparison of T_1's in vesicle and multilayer preparations. T_1 measurements of carbons in multilayers have not been reported previously because of the broad lines and inherently low sensitivity. They have been measured here with the aid of a phosphatidylcholine (PC) phosphatidylethanolamine (PE) mixed system in which the PE is ~60% enriched in carbon-13 at the even carbons of esterified fatty acids.

Methods

Phosphatidylcholine was obtained from fresh egg yolks by the method of Singelton, et al.[6] Phosphatidylethanolamine was obtained by chromatographic separation on silica gel, of the lipids from an E. coli mutant (CY2)[7] grown on 90% enriched 2-^{13}C-sodium acetate (Merck, Sharp and Dohme Canada Limited). The lipids were combined in a 60:40 mole ratio in chloroform and the solvent removed under vacuum. For multilayer preparations the lipids were dispersed by agitation at a concentration of 25% w/v in a deoxygenated D_2O Tris buffer. (.01m Tris, 0.1m NaCl, 0.02% NaN_3 pH 7.4). For vesicle preparation 12% dispersions were sonicated after deoxygenation in a capped tube under nitrogen in a bath type sonicator (Branson Model E) for ~1 hour at 30°C. Vesicles of this composition have been characterized as homogenous preparations of 150Å

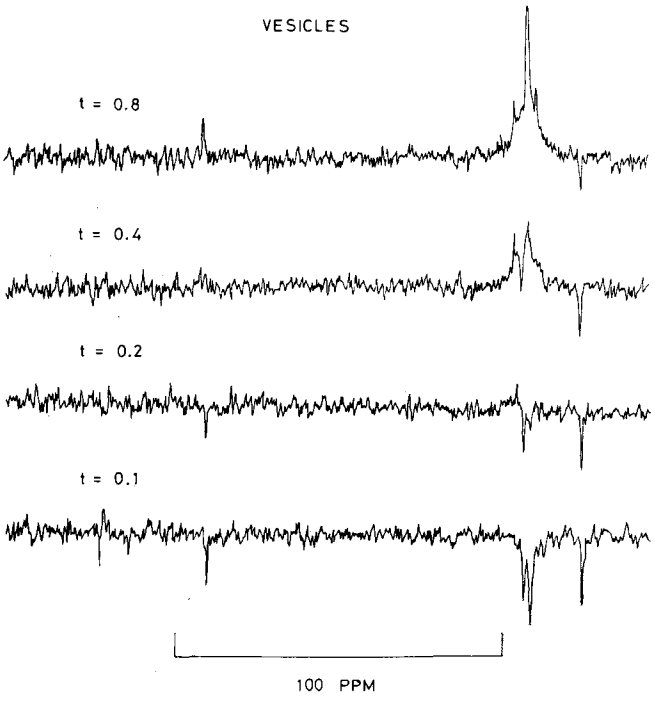

FIGURE 1. ^{13}C Partially relaxed Fourier transformed spectra of vesicles. The lipid composition is PC : PE 60 : 40. Temperature is 50°C. t is the delay between the 180° and 90° transmitter pulses.

radius which show no chemical differences with respect to the dispersions from which they are prepared.[8]

Spectra were run on a Varian CFT-20 spectrometer at several temperatures (8, 28, 48, and 65°C). The values quoted are good to ±2°C. T_1's were determined by the analysis of partially relaxed Fourier transform spectra representing 500 transients each.[9] In consideration of the time devoted to the experiment the delay between successive transients was set at 3 seconds. This is not sufficient time for complete recovery of magnetization for carbons with T_1 > 1 sec. and T_1 values determined directly from a semi log plot are low for methyl and w-2 carbons. The error in T_1 for these carbons can be estimated and a correction has been applied to values reported in Table I. For vesicle systems the values are in substantial agreement with the results of Godici and Landsberger on PC vesicles at 34°C.[5]

Results and Discussion

Representative partially relaxed spectra of vesicles and multilayers are

Table I. Spin Lattice Relaxation Times and Activation Energies.[a]

	Vesicles		Multilayers	
^{13}C Resonance	T_1	E_a	T_1	E_a
C_2	0.32±.04	4.2±.5	0.28±.04	4.8±.5
C_N[b]	0.50±.03	4.8	0.39±.05	4.4
C Double Bond	0.57±.06	4.3	0.31±.05	4.7
C β to Double Bond	0.46±.05	4.0		
C w -2	0.63±.07	4.2	0.43±.05	4.1
C w[c]	(2.2)[d]	4.0	(1.9)	4.0

(a) The sample composition is Egg Yolk PC: E.coli. PE 60:40 prepared as described in methods. The temperature for T_1's is 50°C.

(b) This is a composite resonance of all the unresolved methylenes.

(c) w refers to the terminal methyl carbon. For 80% of the ^{13}C enriched fatty acids w = 16.

(d) Estimated.

given in Figures 1 and 2. Because of ^{13}C enrichment the resonances observed are essentially those of the even numbered carbons in the fatty acids esterified in PE. Assignments should follow those made previously for pure PC vesicles.[10] The vesicle size homogeneity of PC : PE preparations would indicate that PC and PE remain co-dispersed so the T_1 values for methylenes on PE will be representative of methylenes throughout the entire bilayer.

From a comparison of resonance line widths in vesicles and multilayers it is clear that the same qualitative trend found in proton spectra exists; namely a sharp increase in line width increasing radius of curvature. It is therefore apparent that T_2 is dependent on a motion which changes overall bilayer geometry.

In contrast to T_2 values, T_1's for a given resonance are very similar for a vesicle and multilayer. This is apparent from the time course of magne-

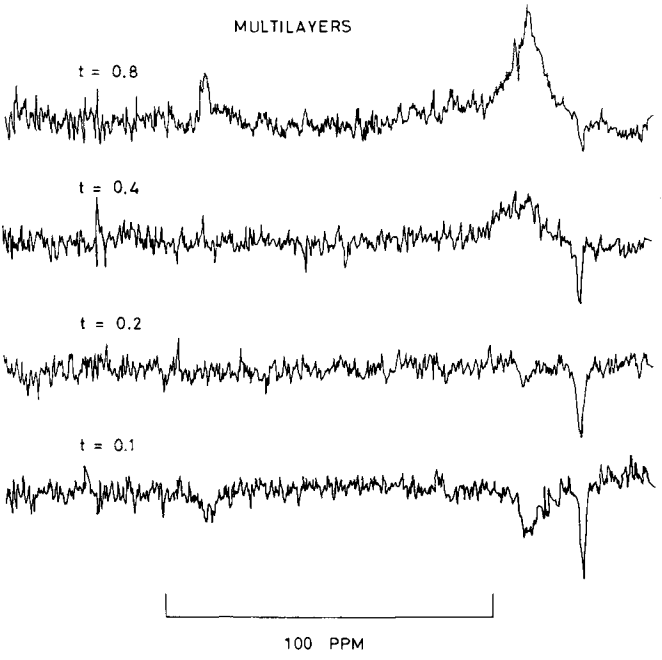

FIGURE 2. ^{13}C Partially relaxed Fourier transformed spectra of multilayers. The lipid composition is PC : PE 60 : 40. The temperature is 50°C. t is the delay between 180° and 90° transmitter pulses.

tization recovery in Figures 1 and 2 or from the T_1 values presented in Table I. That the difference in T_1 and T_2 dependencies are not the result of a change in a single motional correlation time (τ_c) from a value less than $1/w_o$ in vesicles to a value greater than $1/w_o$ in multilayers, where w_o is the Larmor precession frequency, can be ruled out by the fact that temperature studies in both vesicles and multilayers show an increase of T_1 with increasing temperature. It thus seems likely that T_1's and T_2's are characteristic of different motional processes.

The motion that determines T_1 can be characterized in the following ways. First, it is probably anisotropic motion with correlation time, τ_c , $< 1/w_o$ or $< 10^{-8}$ sec. This can lead to the observed nonequivalence of T_1 and T_2 as well as the increase in T_1 with increasing temperature.[11] Second, the motion dominates carbon-13 T_1's in both multilayers and vesicles. This is suggested by the similarity in T_1's and is further supported by the identical activation

energies of motion, 4.5±.5 kcal/mole, calculated from the temperature dependence of the T_1's in both systems. Third, despite dominance in both systems, the motion cannot change greatly in either rate or geometry over wide variations in bilayer curvature. Fourth, the motion must be localized to a small segment of the hydrocarbon chain since there is a pronounced increase in T_1 for methylenes as they become further removed from the lipid head group; $T_{1_{(w-2)}} > T_{1_{(w-n)}} > T_{1_{(C_2)}}$.

A candidate for methylene motion suggested previously, which is consistent with these observations, is the conversion of a three bond segment from a trans-trans-trans to a gauche$^+$ - trans-gauche$^-$ configuration.[1,2] This β coupled isomerization is a substantially anisotropic motion and even with an activation energy of 3-6 kcal is fast enough to have $\tau_c < 10^{-8}$ sec. The motion also retains overall chain geometry so variation in chain packing in going from multilayer to vesicle will have a minimal effect on its existence or rate of occurance. The fact that in a β coupled isomerization only the two central methylenes move, makes its rate sensitive only to local environments which can vary as a function of methylene position on the fatty acid chain.

The unsaturated carbon, constrained in a cis- double bond, can undergo motions analogous to β coupled isomerizations but only one half as many as methylene carbons. Thus, even though there is only one bonded proton causing relaxation, the T_1 for the unsaturated carbon is expected to be approximately equal to the T_1 for methylenes. This is also observed.

The potential dependence of ^{13}C spin lattice relaxation time on the rate of such β coupled isomerizations is significant since this motion is independent of changes in the overall shape of a model or biological preparation. Whether or not this motion retains a great sensitivity to more localized interactions with other membrane constituents must be determined. It is clear, however, that results of ^{13}C T_1 measurements in more complex membrane systems must be discussed in light of a very specific hydrocarbon chain motion rather than a general description of membrane fluidity.

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