CONFORMATIONAL ANALYSIS OF LECITHIN IN VESICLES BY ^{13}C NMR

J.G. Batchelor, J.H. Prestegard
Department of Chemistry, Yale University
R.J. Cushley and S.R. Lipsky
Section of Physical Sciences
Yale University Medical School
New Haven, Connecticut 06520

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Summary. The potential for application of ^{13}C chemical shift data to conformational analysis in membranes has been investigated. Data were collected for egg lecithin in chloroform solution, and in water dispersions as a function of temperature. The ^{13}C shift data can be interpreted in terms of populations of gauche and trans conformers at various points along a hydrocarbon chain.

A phospholipid bilayer is known to be a common structural element of bio-membrane systems.[1] The precise physical state of the hydrocarbon region in this bilayer with its well characterized transition from an ordered state at low temperature to a less ordered one at high temperature[1,2] seems intimately involved with membrane function and cell viability. For this reason, it has recently come under the scrutiny of researchers using a variety of investigative methods.[2,3,4,5] Although informative in many respects, these methods have not been ideal for conformational studies since they are either sensitive to disorder which may be the result of rotational isomerisation about any hydrocarbon bond between the aqueous interface and the point under study, or they are sensitive to the rate of interconversion between different conformations rather than the actual populations of states.

We present here some initial results on phospholipid dispersions using a method based on the interpretation of chemical shift data which may yield more specific information about hydrocarbon conformation. The method relies on the fact that for the ^{13}C resonances of alkane hydrocarbons a 5 ppm upfield shift results when a γ methyl is brought into juxtaposition with the methylene under observation by a gauche conformation of the intervening α-β bond.[6] For the fast exchange limit this enables us to interpret chemical shift as a function of temperature (or other structural perturbation) in terms of alteration in populations of gauche vs. trans rotamers at any site along the hydrocarbon chain for which a resonance can be resolved. The excellent resolution provided by ^{13}C magnetic resonance (CMR) makes this possible even in such complex systems as lecithin vesicles.

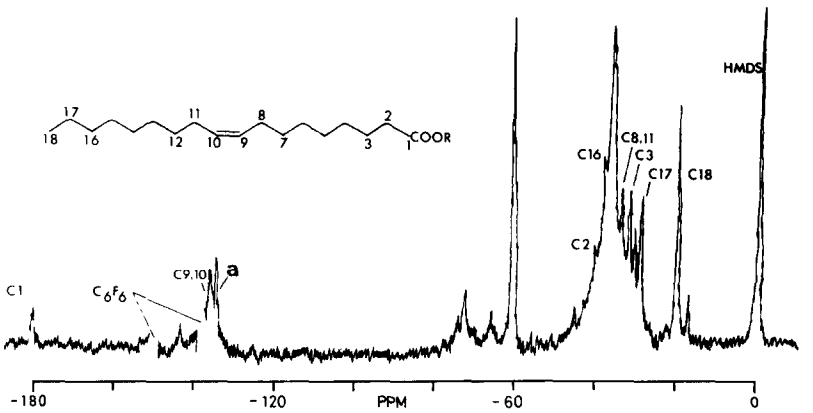

Fig. 1. Natural Abundance ^{13}C spectrum at 50°C of a 20% w/w sonicated lecithin dispersion in H_2O. C_6F_6 peaks have been deleted. We have depicted an oleate residue as one of the two fatty acid chains esterified in a lecithin molecule.

The data presented here are a preliminary test of the sensitivity of the method. They concern not only conformation in saturated chains but also the conformation of bonds near the unsaturated sites in cis-unsaturated acids whose role in bio-membranes has not yet been established.

Experimental

Egg lecithin was prepared by the method of Singleton et al.[7] from fresh egg yolks and stored at -10°C under nitrogen until dispersions were prepared. GC and TLC analysis of a sample after prolonged storage showed the product to be a pure phosphatidyl choline preparation having palmitic, stearic, oleic and linoleic acids in ratios of 4:1:3:2. as major fatty acid components of the diester.

Dispersions of lecithin in water (10-20% by weight) were prepared by agitation on a vortex mixer followed by 1-2 hours sonication under nitrogen in a bath type sonicator. This method should lead to a minimum in decomposition of the natural product while providing a suspension of small closed spherical bilayers (vesicles) suitable for high resolution NMR studies.[8,4]

The spectra were run using a computer controlled Fourier transform ^{13}C spectrometer based on a Bruker 21 Kgauss spectrometer[9] using a 4K data set. Chemical shifts for the various ^{13}C sites were measured as a function of temperature to $\pm$ 0.1 ppm. Data are plotted relative to external 10% HMDS in C_6F_6 at 25°C and have been corrected for variation of bulk susceptibility with temperature.

Spectra were also obtained for a 25% v/v solution of methyl oleate in

chloroform over the range 0°-70°, and for a 20% v/v solution of egg lecithin in chloroform at 46°C.

Results

The natural abundance ^{13}C spectrum of a dispersion of hen egg lecithin in water is presented in Fig. 1. Assignments of resonances arising from carbons in the saturated components of a lecithin preparation have been made previously.[4] Assignments of resonances associated with unsaturated regions have been made by reference to spectra of fatty acids[10] and a series of unsaturated hydrocarbons.[11]

As a matter of convenience peaks have been labelled assuming oleic acid (the principle unsaturate) to be the sole fatty acid constituent. Since chemical shift is largely determined by adjacent functionality, the peaks labelled C18, C17, C16 correspond to all ω, ω-1, ω-2 carbons of fatty acid chains, C9, C10 correspond to all cis-olefinic carbons with saturated chains attached, C8, C11 correspond to all methylenes α to a single unsaturated site. Resonance "a" in Fig. 1 is that of an olefinic carbon β to a second unsaturated site. It is thus characteristic of the polyunsaturates present.

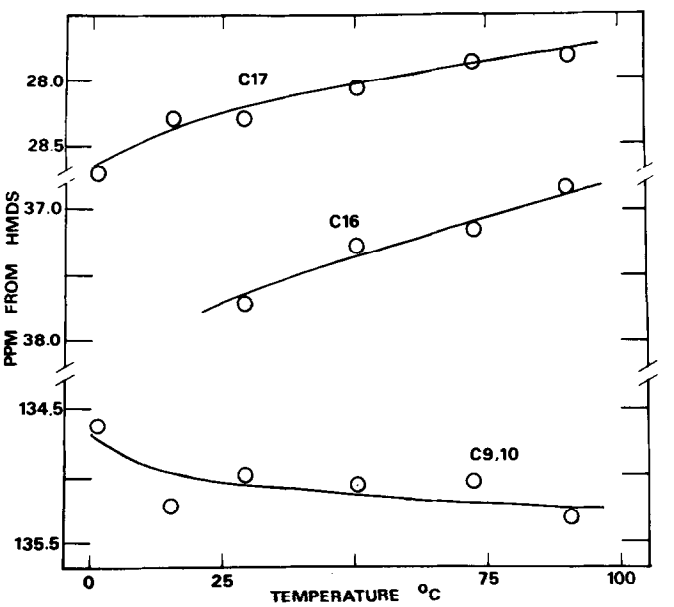

Fig. 2. ^{13}C shifts downfield from external HMDS 10% in C_6F_6 for selected resonances of lecithin in vesicles as a function of temperature.

Fig. 2 presents the temperature dependence from 0° to 90°C of several peaks illustrating typical saturate and unsaturate behavior. With the notable exception of the olefinic (C9, C10) peak, a gradual downfield shift is noted as temperature decreases and the lecithin approaches a more ordered state. The temperature dependence of the resolvable methylenes, not illustrated in Fig. 2, qualitatively follows that of the C17 and C16 peaks. The methylene shifts of methyl oleate with temperature are qualitatively similar to those observed for lecithin vesicles but are of smaller magnitude. C16, for example, shifts upfield 0.2 ppm in going from 6° to 70°C. The C9, C10 olefinic shifts with temperature, on the other hand, are less than experimental error. The absolute values of the chemical shifts of the methylenes of methyl oleate and lecithin in chloroform solutions are in general slightly upfield from the corresponding shifts in lecithin vesicles at similar temperatures. This difference is particularly marked for the C3 carbon for which it is 0.8 ppm.

Discussion

^{13}C shifts of resonances arising from carbons in an afunctional hydrocarbon environment, such as that for most of the carbons in the interior of the bilayer can be attributed to a single source. Our own work with model compounds, 2-methyl-2-hexene and 2,5-dimethyl-2-hexene, shows that although solvent effects on methyl groups can be significant, the effects on methylenes are negligible and effects on methine carbons are ≤ 0.2 ppm over a range of solvents. Shifts for the terminal methyl of heptanoic acid in aqueous solution in going from pH 7.3 to pH 1.6 are no more than 0.3 ppm indicating charge effects to be small at this distance from the head group. Shift variations due to temperature dependent solvation or electric field effects for most methylenes in the sheltered environment of a bilayer should therefore be insignificant. Remote group magnetic anisotropy effects will not exceed the minimal effects seen in proton spectra of lipid dispersions. The only sizable effect, therefore, is that linked to rotational isomerisation about carbon-carbon bonds. Electron delocalization, possible when a γ methyl is brought near a second methyl by an intervening *gauche* bond, leads to a predicted 4.8 ppm shift to a higher field.[6] If a similar effect dominates the methylene temperature dependence of chemical shifts for lecithin and methyl oleate, the results are easily explained. For C-C bonds in both liquid hydrocarbon solutions and in the hydrocarbon portion of a fully saturated lipid bilayer the *trans* conformer is usually the low energy form. Thus as temperature is lowered and *trans* states are populated to a greater extent one would expect a downfield shift. For a liquid *n*-alkane with a 0.5 kcal separation

of gauche and trans conformers,[12] a 0.2 ppm shift is predicted in going from 0°C to 70°C. This is consistent with the data for the terminal methylenes of methyl oleate in chloroform. The more pronounced shifts at lower temperatures in lecithin vesicles can be attributed to nearing the broad cooperative phase transition centered about -10°C.[2].

The shifts of the unsaturated carbons, (C9, C10), are perhaps more interesting. If we ignore the difference in electronic configuration at the unsaturated carbon in applying a formula developed by Grant and Cheney[6] for saturated systems, we would expect a gauche conformer about the 7-8 or 11-12 bonds to cause a 1-2 ppm upfield shift of the C9, C10 resonance. This is supported by the 1 ppm shift of the C3 resonance in 2,5-dimethyl-2-hexene upfield of that in 2-methyl-2-hexene which has fewer gauche interactions. One might also expect the temperature dependence of the unsaturated carbon resonance (C9, C10) to parallel that of methylenes in saturated compounds. However, energy differences for rotational states of bonds β to a cis-unsaturate are very small[12] and thus the different states will be nearly equally populated at all temperatures. Thus the lack of temperature dependence for these resonances in the model compounds and in methyl oleate is not surprising. The large downfield shift of the unsaturated carbon resonance in lecithin vesicles (see Fig. 2) suggests that a gauche orientation about the 7-8 or 11-12 bond is preferred in this system as the phase transitio is approached. This preference in the bilayer must be due to conformational restrictions imposed by the bilayer rather than any intramolecular effect. An inspection of molecular models shows that a 7-8 or 11-12 gauche bond conformation makes oleic acid a nearly linear molecule. We would expect a chain with such a conformation to pack more closely into the hydrocarbon region than the non-linear trans conformer. These considerations will become more important as a transition to an ordered state is approached.

Even above the phase transition, bilayer imposed conformational restrictions will exist. ^{13}C shifts for most methylenes are about 0.3 ppm further downfield in lecithin vesicles than in either lecithin or methyl oleate solutions in chloroform at 45°C. This indicates a greater trans population in vesicles, since solvent shifts for methylenes in such systems are negligible (see above). The effect is greatest (0.8 ppm) for C3 which is located near the aqueous interface.

At present we have no absolute values for the ^{13}C shifts of exclusively trans and exclusively gauche conformers at the various sites of an oleate chain and so we cannot yet quantify the proportions of each conformer in our samples. More quantitative results should be obtained in experiments now under way on membrane systems whose phase transitions are completely within our accessible

temperature range. However, our present qualitative results have provided insights into hydrocarbon conformation in bilayers and have illustrated the potential ^{13}CMR offers for conformational analysis in membrane systems. In particular, the tendency toward a gauche bond β to a cis-unsaturate lends support to the recent suggestion[3] that gauche-gauche pairs exist in the fluid state of fully saturated systems. The existence of such structures may be a significant factor in many membrane associated phenomena.

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