EFFECT OF VISCOSITY ON THE WIDTH OF THE METHYLENE PROTON MAGNETIC RESONANCE LINE IN SONICATED PHOSPHOLIPID BILAYER VESICLES

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Received 30 December 1977

1. Introduction

Several experimenters [1–4] have reported that the methylene proton magnetic resonance (PMR) linewidth in small single bilayer vesicles is constant for a wide range of viscosities. They have consequentially inferred that the only molecular motions responsible for narrowing of the methylene line in vesicles are rapid conformational isomerizations and other motions of the hydrocarbon chains rather than the diffusion of phospholipid molecules around the vesicles or vesicular Brownian tumbling, which should be a function of the viscosity of the suspending medium. Because the methylene PMR line is two orders of magnitude broader in large multilamellar phospholipid dispersions than in small vesicles, it was deduced that the degree of disorder resulting from acyl chain isomerizations is substantially greater in vesicles than in multilamellar dispersions. However, in a recent phosphorus magnetic resonance investigation [5], the phosphorus line from the headgroups of dipalmitoyl lecithin and egg yolk lecithin (EYL) in small vesicles was shown to exhibit a dependence on the medium viscosity which was entirely consistent with the phosphorus line being narrowed by vesicular Brownian tumbling and by phospholipid diffusion. Furthermore, values of the diffusion constant D for the phospholipid molecules calculated from the data were in good agreement with independent measurements of the diffusion constant in sonicated and unsonicated phospholipid dispersions by other methods [4,6–9].

In contradiction to published results we present in this communication clear experimental evidence that the PMR methylene linewidth in small vesicles is dependent on the viscosity of the suspending medium and we extract a diffusion constant for egg yolk lecithin molecules in bilayers in agreement with that obtained by other techniques. We shall conclude that the difference in widths between the methylene line in vesicles and in multilamellar dispersions is determined by Brownian tumbling and lateral diffusion and that there is no need to invoke large additional narrowing mechanisms.

2. Materials and methods

Egg yolk L-α-phosphatidylcholine (type III E) was obtained from Sigma. D_2O (99.8% D) was purchased from Merck Sharp and Dohme, Canada Ltd. Glycerol (analytical reagent from Mallinckrodt Chemical Works) was used since perdeuterated glycerol from Merck Sharp and Dohme was found to contain a $(CH_2)_n$ impurity.

EYL in hexane solution was dried under a stream of nitrogen, redissolved in chloroform, dried under nitrogen and further dried under vacuum for 15 min to remove the last traces of solvent. The lipid was dispersed in D_2O to give 5% w/w. The dispersion was sonicated with the ½ in. probe of a Bronwill Biosonik for 1 h at 18–20°C under nitrogen. After the sample had been centrifuged for 45 min at 5°C at 35 000 rev/min in a Spinco 40 rotor, the clear central portion was extracted and used for the PMR measurements. The distribution of vesicle sizes was determined by electron microscopy.

Vesicle suspensions with different viscosities were

prepared by adding measured amounts of glycerol. When vesicle suspensions were added back to the glycerol/vesicle mixtures, the glycerol-induced line broadening was found to be reversible. The samples were mixed thoroughly using a vortex mixer for several minutes. The viscosity of D_2O/glycerol mixtures (containing no vesicles) was measured with an Ostwald viscometer.

The PMR measurements were obtained with a Nicolet TT-23 spectrometer operating at 100 MHz using a 5 kHz spectral width with no filtering, a 20 μs pulse width and a frequency offset of 2.5 kHz from the PMR of HDO. The fidelity of the resulting lineshapes was checked by accurately verifying the Lorentzian form of the PMR of a sample of H_2O to which paramagnetic ions were added to broaden the PMR line to a width of 30 Hz.

3. Results and discussion

In this communication we shall not discuss the rather complicated, non-Lorentzian shape of the methylene resonance line [10,11]. We shall simply take advantage of the fact that the shape associated with the dipolar broadening of the methylene line is independent of the rate of reorientation of the phospholipid molecules in the vesicles. Hence, the methylene linewidth $\Delta\nu$ is linearly related to the reorientational correlation time τ_v which for a vesicle of radius R in a medium of viscosity* η at temperature T is given by [13]

$$\frac{1}{\tau_v} = \frac{3kT}{4\pi\eta R^3} + \frac{6D}{R^2} \qquad (1)$$

where k is the Boltzman constant and D is the lateral diffusion constant. The first term describes reorientation of the vesicle as a unit and the second describes diffusion of the molecules around the surface of the vesicle.

Making no assumptions about the details of the vesicle methylene lineshape we can write

$$\Delta\nu - C = A\,\tau_v \qquad (2)$$

where C is the intrinsic methylene linewidth arising from the distribution of chemical shifts and the inhomogeneity of the applied magnetic field (C = 4 Hz estimated from the PMR spectrum of EYL in deuterated chloroform) and $\Delta\nu$ is the full width at half height in Hertz. For a single spin undergoing simple motional narrowing [14], the constant A would be the rigid-lattice second moment M_2; however, the methylene protons are a multiple spin system in which all spins are not equivalent and the problem is considerably more complex [10,11].

In fig.1, we show PMR spectra for EYL vesicles in

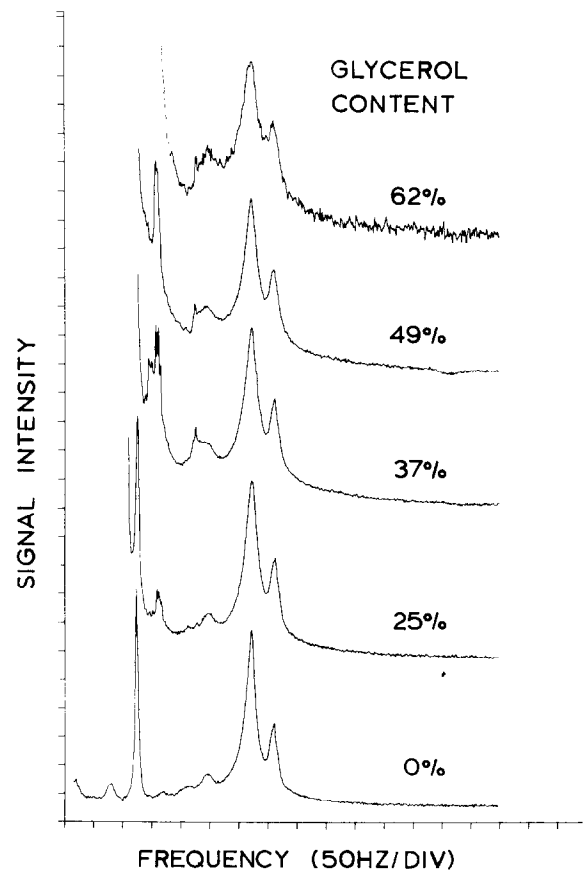

Fig.1. Proton magnetic resonance spectra for egg yolk lecithin vesicles suspended in glycerol/water mixtures. The second line from the right is from the methylene protons and the large absorption on the left in the top 4 spectra is due to glycerol.

*For dilute vesicle suspensions where vesicle–vesicle interactions are negligible the viscosity of the suspending medium, η, determines the rate of reorientation of the vesicles [12]

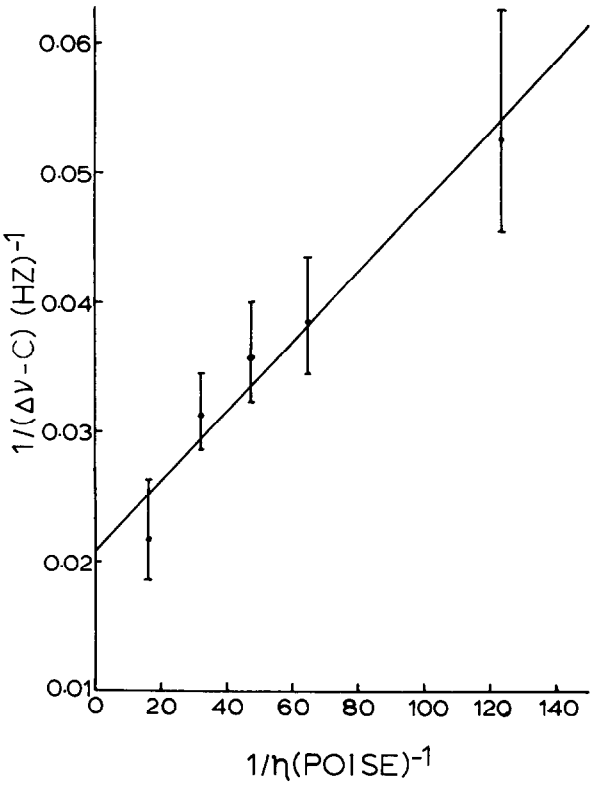

Fig.2. The inverse of the methylene linewidth (corrected for the contribution from the intrinsic width) is plotted as a function of the inverse of the medium viscosity.

5 different D_2O/glycerol mixtures spanning a factor of more than 7 in viscosity. A plot of $1/(\Delta\nu - C)$ against $1/\eta$ taken from these spectra yields a straight line (fig.2) for which the ratio of slope to intercept is:

$$\text{slope/intercept} = \frac{kT}{8\pi D R_{\text{eff}}} \qquad (3)$$

where we have replaced R by R_{eff} to take into account the fact that not all vesicles have the same radius. For a narrow distribution of vesicle sizes, R_{eff} may be identified with the average radius to a first approximation. From the line fitted by a least square value analysis to the data in fig.2 and a R_{eff} value 1.9×10^{-6} cm obtained from a statistical analysis of electron micrographs of our vesicle sample, the lateral diffusion constant for the phospholipid molecules in EYL vesicles was found to be 7×10^{-8} cm^2/s at 31°C. Considering the inaccuracies inherent in our measurement of D, this value is in reasonable agreement with that obtained by a similar procedure using phosphorus magnetic resonance [5] indicating that the motional narrowing mechanism for vesicle lineshapes is the same for both the headgroup and the hydrocarbon chains in EYL. Our value for D is also within the range of values obtained by other techniques (table 1). The advantages and disadvantages of using glycerol/vesicle mixtures for the measurement of diffusion constants are discussed [5].

Table 1
A list of measurements of lateral diffusion in egg yolk lecithin

Diffusion constant ($\times 10^8$ cm^2/s)	Temp. (°C)	Method	Ref.
1.8 ± .6	25	EPR in multilayers	[6]
1	50	EPR in multilayers	[7]
1.8	30	^{31}P NMR in multilayers	[7]
.9	20	^{1}H NMR in vesicles	[4]
2.7	50	^{31}P NMR in vesicles	[5]
2.1	24.2	^{1}H NMR in cubic phase	[9]
50	25	Fatty acid probe in multilayers using a radio tracer technique	[15]
17–24	25	Fluorescence technique in black lipid membrane	[16]
7	31	^{1}H NMR in vesicles	this study

In conclusion, we have clearly demonstrated that the methylene PMR linewidth in small phospholipid vesicles depends both on the rate of vesicular tumbling and on phospholipid lateral diffusion.

Acknowledgements

This research was supported by the National Research Council of Canada.

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