Biochimica et Biophysica Acta, 603 (1980) 63—69
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BBA 79019

EFFECTS OF TUMBLING AND LATERAL DIFFUSION ON PHOSPHATIDYLCHOLINE MODEL MEMBRANE ^{31}P-NMR LINESHAPES

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(Received May 1st, 1980)

Key words: ^{31}P-NMR lineshape; Phosphatidylcholine; Vesicle tumbling; Lateral diffusion; (Model membrane)

Summary

Two factors determining the isotropic motional averaging of NMR spectra obtained from lipids in model and biological membranes systems are particle tumbling and lateral diffusion. The influence of these motions (of which the magnitudes are determined by the medium viscosity and temperature) on the ^{31}P-NMR spectra arising from unilamellar dioleoyl phosphatidylcholine vesicles of a defined size are examined. It is shown that the lineshapes obtained are in good agreement with those predicted by the theory of motional narrowing. These results are discussed with regard to order parameter determinations and polymorphic phase identifications as obtained by NMR techniques.

In systems where isotropic motional averaging effects arising from phospholipid lateral diffusion or membrane tumbling are negligible, ^{31}P-NMR techniques may be employed to advantage to gain information on the local order and conformation in the phosphate region of the polar headgroup [1] as well as on the polymorphic phase (e.g., bilayer or hexagonal) [2]. In such situations, the spectra obtained exhibit broad, asymmetrical lineshapes reflecting the restricted anisotropic motion of the phospholipid molecules [1,2].

Alternatively, in small systems such as sonicated vesicles, narrow symmetrical 'high resolution' ^{31}P-NMR spectra are observed [1—3]. These spectra reflect isotropic motional averaging arising from lateral diffusion of phospholipids around the vesicle and Brownian tumbling of the entire vesicle [3],

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both of which are sufficiently rapid such that the NMR spectra are in the motionally narrowed regime.

Clearly, for phospholipid systems of intermediate size, situations will be encountered for which the ^{31}P-NMR spectrum is intermediate between a narrow symmetrical and a broad asymmetrical lineshape. It is important to understand such spectra as previous studied [4–9] lead us to suspect that certain characteristics of the NMR behaviour of systems of major interest (including model, biological and reconstituted membrane systems) correspond to such an intermediate situation. Therefore, in this study we have examined the ^{31}P-NMR behaviour of unilamellar dioleoyl phosphatidylcholine vesicles of defined sizes where the rotational averaging is varied in a controlled manner. We find that the lineshapes obtained correspond closely to those predicted by the theory of Freed et al. [10,11], and that lateral diffusion can markedly affect the ^{31}P-NMR spectra obtained from relatively large (e.g., 2000Å diameter and even larger) membrane systems.

Unilamellar vesicles of defined sizes were prepared by a modification [12] of the ethanol-injection method [13] from chromatographically pure 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (dioleoyl phosphatidylcholine) which was synthesized according to the method of Warner and Benson [14]. An ethanol solution (0.4 ml) of dioleoyl phosphatidylcholine (12.5–100 mg/ml) was injected at 25°C with a motor-driven syringe at a rate of 0.050 ml/min into 10.0 ml of a stirred 100 mM NaCl, 10 mM Tris-HCl, 0.2 mM EDTA, pH 7.0 buffer solution. Ethanol was removed by dialyzing the vesicles three times against 1 l of the buffer. The vesicle sizes were measured (after dilution of aliquots of this solution with buffer) by angle-dependent light-scattering techniques using a Fica light-scattering photometer and techniques as described by Kremer et al. [12]. The radius of the vesicles [12] was obtained by extrapolation to $\theta = 0°$ of plots of the Rayleigh ratio at angle θ against $\sin^2\theta/2$ [12,15]. As observed for dimyristoyl and dipalmitoyl phosphatidylcholine these plots were linear over a large range of θ, indicating that these vesicles are fairly monodisperse [12,15]. Liposomes (100 mg lipid/ml) were prepared in the same buffer as described before [16].

The size of the vesicles prepared by the ethanol-injection method can be varied by varying the lipid concentration in ethanol [12,15]. This is shown in Fig. 1 for dioleoyl phosphatidylcholine. The radius of the vesicles varies from 415 to 980Å for lipid concentrations of 12.5 to 100 mg/ml ethanol. Larger vesicles could not be prepared because of the limited solubility of the lipid in ethanol.

^{31}P-NMR measurements were performed using gated high-power proton decoupling at 81 MHz on a wide-bore Bruker WP 200 spectrometer employing 20-mm diameter NMR tubes and 10-ml samples. The large tube diameter was essential to obtain reasonable signal-to-noise ratios for some of the samples which could not be concentrated. Concentrating the vesicles by centrifugation or ultrafiltration leads to aggregation or fusion of the vesicles (de Kruijff, B., unpublished observations). The vesicles were diluted with 10% of a ^2H_2O analog of the buffer. The medium viscosity was varied by slowly adding glycerol to the samples while stirring. The pH of the solution was not affected by the addition of glycerol. A sweep width of 20 kHz and 4K data points were used.

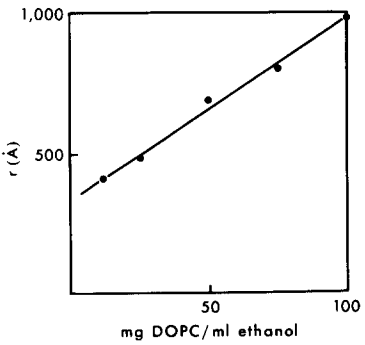

Fig. 1. Effect of the concentration of dioleoyl phosphatidylcholine (DOPC) in ethanol on the radius of the vesicles prepared by the injection method.

Typically, 1000—25 000 transients were recorded with a 1 s interpulse time employing 90° radiofrequency pulses. An exponential multiplication corresponding to a 50 Hz line broadening was applied to all free induction decays. Spectra recorded on a given sample at various temperatures were fully reversible.

The correlation time, τ_c, for isotropic rotational motion of a spherical vesicle is related to the vesicle size by:

$$\frac{1}{\tau_c} = \frac{6}{r^2}\,(D_t + D_{diff})$$

where $D_t = kT/8\pi r\eta$ (η is the medium viscosity) is the tumbling-dependent part describing Brownian rotational diffusion and D_{diff} is the rate of lateral diffusion of the lipid molecules in the bilayer [3,18]. The τ_c values can be estimated from the known medium viscosities and the diffusion rates of dioleoyl phosphatidylcholine in vesicles at various temperatures as obtained by Galla et al. [17] (data obtained by pyrene eximer formation, Table I). The ^{31}P-NMR spectra of the largest vesicles (980Å radius) at various temperatures and glycerol concentrations are presented in order of increasing τ_c values in Fig. 2G—K. For small τ_c values a symmetrical, rather narrow resonance is observed (Fig. 2G). As τ_c is increased the ^{31}P-NMR spectra become broader and increasingly asymmetrical. The largest τ_c value (Fig. 2K) gives rise to a spectrum which is similar to that observed for the dioleoyl phosphatidylcholine liposomes (Fig. 2L). Glycerol at the concentrations employed here did not affect the ^{31}P-NMR spectrum of the liposomes. Furthermore, in the case of vesicles, the ^{31}P-NMR spectra of the vesicles in glycerol at higher temperatures were very similar to spectra obtained without glycerol but recorded at a temperature such that the τ_c value was identical. These and other literature data [3,18] strongly suggest that glycerol only affects vesicle tumbling. Additionally, the ^{31}P-NMR spectra of the smaller dioleoyl phosphatidylcholine vesicles were very similar to those shown in Fig. 2 when they were recorded under conditions such that τ_c was identical to the values shown in Fig. 2.

Recently, Campbell et al. [11] published a paper describing the slow motional ^{31}P-NMR lineshapes of phospholipids. Their analysis is based on an earlier work [10]. Here we use a computer program based on the theory of

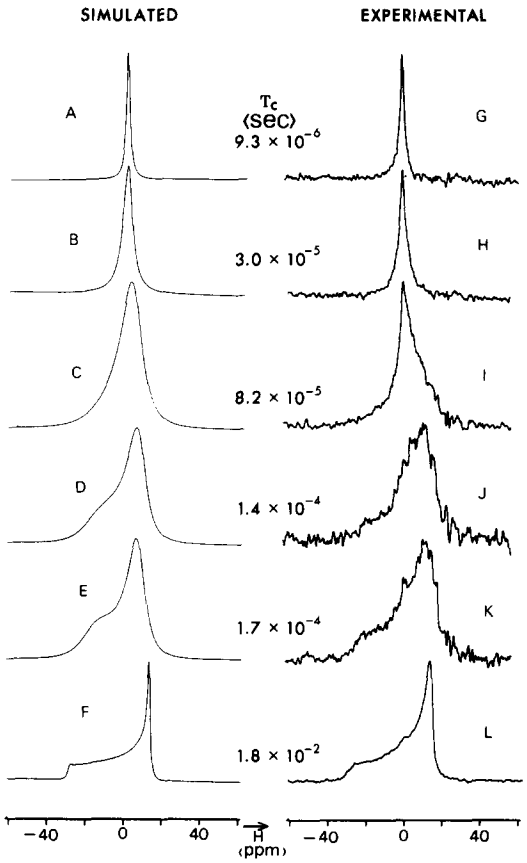

Fig. 2. Simulated (A—F) and experimental (G—L) 80MHz ^{31}P-NMR spectra of dioleoyl phosphatidylcholine vesicles for different values of τ_c as indicated. The spectra were simulated using $(\sigma_\parallel - \sigma_\perp) = 3550$ Hz, and the orientationally independent Lorentzian full line width at half height = 60 Hz. For spectra G—K, the values of τ_c were calculated from the equation in the text using $R = 980$Å, η from tables of viscosity for water and for water/glycerol mixtures (Handbook of Chemistry and Physics), and D_{diff} from Table I of Ref. 17. For spectrum L, τ_c corresponding to an arbitrarily large radius of 2.6 μm was used. The experimental spectra were measured under the following conditions: G, 60°C; H, 25°C; I, 0°C; J, −10°C, 30% glycerol; K, −15°C, 30% glycerol; and L, 30°C, unsonicated liposomes.

Freed et al. [10] to simulate the measured spectra using no adjustable parameters. This analysis assumes an axially symmetrical chemical-shift tensor and reorientational motion which is governed by an isotropic diffusion equation. First, a rigid lattice spectrum was simulated to give values of $(\sigma_\parallel - \sigma_\perp)$ (Fig. 2F). Throughout, we use a Lorentzian line broadening (full width at half height) of 60 Hz; this is consistent with the 50 Hz used in the experimental spectra plus an arbitrary 10 Hz natural linewidth. Using these parameters, ^{31}P-NMR lineshapes were generated for the τ_c values calculated for the various experimental conditions. The calculated (Fig. 2A—E) spectra obtained with no adjustable parameters are in reasonable agreement with the experimental spectra (Fig. 2G—K).

The reason for the small discrepancies between the experimental and the theoretical spectra might be caused by vesicle size heterogeneity and by the

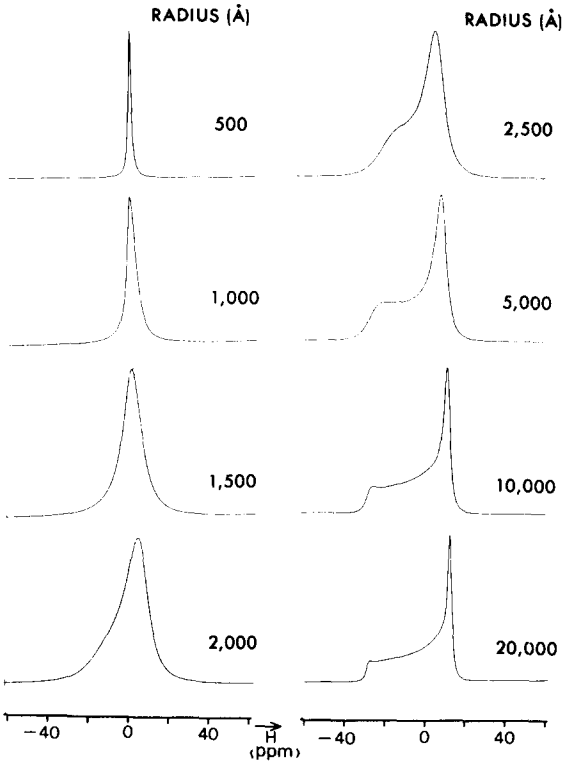

Fig. 3. Simulated ^{31}P-NMR spectra of dioleoyl phosphatidylcholine vesicles of different sizes at 30°C. The spectra were simulated using $(\sigma_{\parallel} - \sigma_{\perp}) = 3550$ Hz, $\eta = 0.008$ P, orientationally independent Lorentzian full line width at half height = 60 Hz, $D_{diff} = 6.2 \cdot 10^{-7}$ cm^2/s, and R as indicated.

fact that no angle-dependent T_2 was used in the simulations. The experimental liposomal spectrum (Fig. 2L) apparently is already slightly motionally narrowed.

In order to obtain insight into the spectra of larger (greater than 980Å radius) vesicles, we repeated the calculations for dioleoyl phosphatidylcholine vesicles of various sizes at 30°C using the same parameters as used in Fig. 2. For vesicle sizes up to approx. 1500Å radius, the lineshape is symmetrical and becomes increasingly broader with increasing size (Fig. 3). Between 1500 and 2000Å radius, the spectrum becomes asymmetrical and at 2500Å radius exhibits a pronounced 'bilayer' lineshape. It is of interest to note, however, that even in the 5000—10 000Å radius range the spectrum is still sensitive to the size.

These data demonstrate that in ^{31}P-NMR studies on (model) membranes, knowledge of the vesicle size is essential and that the use of the ^{31}P-NMR lineshape for detection of bilayer structure [2] is restricted to systems with radii of 2000—3500Å (for lateral diffusion rates of the order of $5 \cdot 10^{-7}$ cm^2/s).

The chemical shift anisotropy, $\Delta\sigma$ (the distance between the low-field shoulder and the high-field peak), and the width of the ^{31}P-NMR spectrum ($\Delta\nu_{1/2}$, full width at half height) are commonly used to obtain information

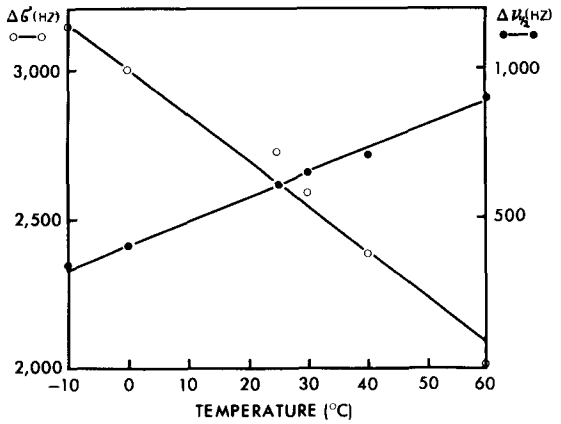

Fig. 4. Temperature dependence of $\Delta\sigma$ and $\Delta\nu_{1/2}$ obtained from the simulated ^{31}P-NMR spectra of dioleoyl phosphatidylcholine vesicles with a radius of 5000Å. Other parameters for the simulation are as indicated in the caption to Fig. 2.

on the local order of the phosphate region of the lipid molecule in the bilayer [1]. Since the data and calculations presented here strongly suggest that even for 5000—10 000Å radius vesicles the lineshape is dependent on τ_c, we decided to measure $\Delta\sigma$ and $\Delta\nu_{1/2}$ from the calculated spectra of 5000Å radius (which is a typical size for an unsonicated liposome) dioleoyl phosphatidylcholine vesicles at various temperatures. The results plotted in Fig. 4 demonstrate that the temperature dependence of D_{diff} (which is the major source of isotropic averaging) yields values both $\Delta\sigma$ and $\Delta\nu$ which are temperature dependent. We therefore suggest that the use of these parameters in calculations of the order parameter(s) of the phosphate group is potentially misleading, particularly when comparisons are made between different membrane systems of unknown sizes. Thus, the observations that in reconstituted lipid-protein systems $\Delta\sigma$ is decreased [7—9] and $\Delta\nu_{1/2}$ is increased [8,9] compared to that observed in protein-free liposomal systems could well result from the smaller size of the reconstituted systems and not indicate a change in local order.

It should also be noted that these considerations apply with equal force to order parameters obtained from ^{2}H-NMR studies of model and reconstituted systems containing ^{2}H-labelled lipids. An unambiguous interpretation of changes in quadrupolar splitting induced by changes in temperature, protein content, etc., in terms of changes in local order requires a precise knowledge of the size of the system and the lateral diffusion rates of the lipids.

Acknowledgements

We would like to thank Drs. P.A.M.M. Aarts and J.M.H. Kremer for their help in the light-scattering experiments. This work was supported by the N.S.E.R.C., M.R.C. and Canadian Heart Foundation. B. de K. is a visiting scientist of the M.R.C. and Canadian Heart Foundation (1979—1980). P.R.C. is a research scholar of the M.R.C.

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