FREQUENCY DEPENDENCE OF ^{31}P NMR LINEWIDTHS IN SONICATED PHOSPHOLIPID VESICLES: EFFECTS OF CHEMICAL SHIFT ANISOTROPY

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1. Introduction

Phosphorus nuclear magnetic resonance (^{31}P NMR) is an increasingly important physical technique for the elucidation of structural features of phospholipid bilayer membranes [1–5]. In sonicated vesicles, however, the chemical shift differences between different classes of phospholipids are of approximately the same magnitude as the widths of the resonances themselves [5,6]. For many applications it is desirable to find conditions which optimize the resolution of these signals.

In general, the resolution of chemically-shifted resonances is improved by increasing the field strength, as the separation of the resonances increases linearly with the field strength, while the width of the resonances is usually field-independent. The ^{31}P NMR spectrum from unsonicated phospholipid dispersions is, however, dominated by the chemical shift anisotropy of the phosphate group [2,4]. It might therefore be expected that the ^{31}P NMR linewidths in the sonicated phospholipid bilayer systems would contain a term which arises from the modulation of this chemical shift anisotropy by the isotropic tumbling of the vesicles. In this case, the ^{31}P NMR linewidths of sonicated bilayer vesicles would broaden as the field is increased, decreasing the resolution at higher field strengths.

This communication describes some experimental results which allow an estimate of this effect, and discusses the implications.

2. Materials and methods

Synthetic β-γ-dipalmitoyl L-(3) lecithin (DPL) and brain sphingomyelin were obtained from Koch-Light Laboratories, Ltd., Colnbrook, Bucks, England; and egg lecithin was obtained from Lipid Products, South Nuffield, England. All lipids were used without further purification. Praseodymium chloride was prepared from the oxide (obtained from Koch-Light Laboratories) by titration with hydrochloric acid. All other reagents were analar grade and were used without further purification. Phospholipid vesicle preparations were obtained by 30 min sonication under nitrogen of a 50 mg/ml phospholipid: D_2O mixture is a Dawes sonicator system (power level 3) in the presence of 25 mM Tris (hydroxymethyl) amino methane (pH 7.0) and 5.0 mM EDTA.

^{31}P NMR spectra were recorded at 36.43 MHz on a Bruker HX 90 spectrometer which was interfaced with a Nicolet B-NC 12 computer and equipped with temperature control and broad-band proton noise decoupling. ^{31}P NMR spectra were recorded at 129 MHz on an instrument constructed in this laboratory, which was interfaced with a Nicolet B-NC 12 computer and equipped with temperature control, but not with proton decoupling. Details of the latter spectrometer have been reported elsewhere [7]. Both instruments

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were operated exclusively in the Fourier Transform mode. Accumulated free induction decays were obtained from up to 1000 transients, employing a 2 sec. interpulse time.

3. Results and discussion

The ^{31}P NMR linewidths of sonicated dipalmitoyl lecithin vesicles are remarkably dependent on temperature. Below the phase-transition temperature (~ 41°C) the linewidths decrease sharply as the temperature is increased, while above the phase transition the linewidths are essentially independent of temperature. At 129 MHz the observed linewidth narrows from 100 Hz to 43 Hz between 30°C and 50°C, while at 36.4 MHz the observed linewidths narrow from 22 Hz to 8 Hz (see fig. 1). At 50°C and 36.4 MHz the linewidths are narrow enough to resolve two resonances, separated by ~5 Hz, each with a width of ~4 Hz. These two resonances may be assigned to the phospholipid molecules on the inside and the outside of the bilayer vesicles respectively, as the addition of praseodymium chloride to the external aqueous phase broadens and shifts only one of the resonance in the composite peak (the low-field resonance) to lower field values. Resolved resonances of similar widths and separation have also been observed for the inside and outside phospholipids of sonicated egg-yolk vesicle preparations (36.4 MHz, 30°C).

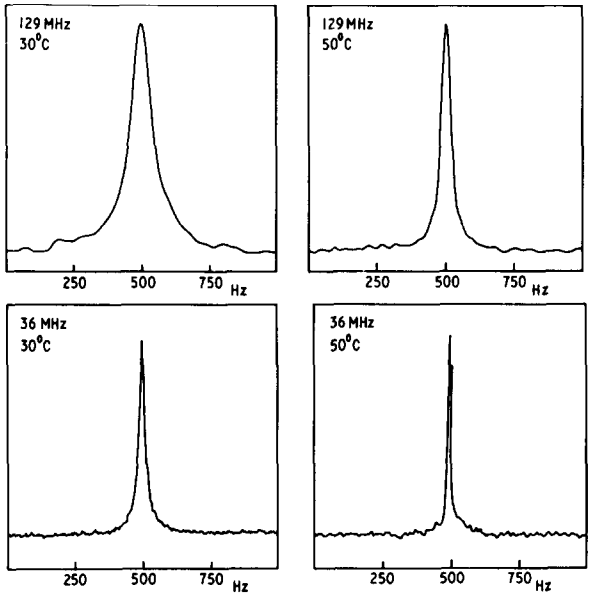

Fig. 1. ^{31}P NMR spectra of sonicated dipalmitoyl lecithin (50 mg/ml) at 36 MHz and 129 MHz. All spectra have the same scale in Hz.

Fig. 1 also shows that the ^{31}P NMR linewidths from sonicated dipalmitoyl lecithin are strikingly dependent on the magnetic field strength. Even after correction for the unresolved chemical shift difference between the inside and the outside phospholipids (and the lack of proton decoupling at 129 MHz), the linewidths increase by roughly a factor of five between 36.4 MHz and 129 MHz, both above and below the phase transition. This frequency dependence could not arise from different environments in the plane of the membrane giving rise to a heterogeneity of chemical shifts, as the rapid lateral diffusion would effectively average these out [8]. This implies that the relaxation mechanism which determines the ^{31}P NMR linewidths must be field dependent. The only plausible relaxation mechanism which is field dependent arises from the chemical-shift anisotropy of the phosphate group which is modulated by the isotropic tumbling of the vesicles. This mechanism will introduce a term $\Delta\gamma_{CSA}$ into the linewidth, which is given by

$$\Delta\gamma_{CSA} \propto H_0^2 (\sigma_{\parallel}-\sigma_{\perp})^2 \tau_R,$$

where $\sigma_{\parallel}-\sigma_{\perp}$ is the chemical shift anisotropy of the phosphate group, H_0 is the applied magnetic field, and τ_R is the tumbling time of the vesicles [9]. The total linewidth will be the sum of a dipolar term, which is independent of the applied magnetic field, and the chemical-shift anisotropy term, which depends on the square of the field. The relative magnitudes of the two terms may then be determined by the observed frequency dependence. From our observations, chemical shift anisotropy can be estimated to contribute ~30% of the total observed linewidth at 36.4 MHz and ~86% of the total observed linewidth at 129 MHz.

The field dependence of the linewidths has several important consequences. First of all, it implies that the sensitivity will be optimum at ~50 MHz, and will decrease as the field is raised. This is in contrast with the situation in proton NMR, where the sensitivity continues to increase as the field is raised. Also, it implies that the resolution of chemically shifted lines from different phospholipids will be optimum at ~50

MHz, since above that frequency the linewidths begin to increase as the square of the field, while the separation between the two lines increases only linearly with the field. In the case of larger vesicles (with a correspondingly reduced tumbling rate τ_R^{-1}) the contribution to the linewidth from chemical shift anisotropy will be larger, and the optimum frequency for resolution will be lower. However, a positive aspect is that the field dependence of phosphorus linewidths may be used to estimate chemical shift anisotropies if the relevant correlation times are known.

Fig. 2a shows the ^{31}P NMR spectrum of a co-sonicated mixture of dipalmitoyl lecithin and sphingomyelin at 36.4 MHz. The sphingomyelin resonances are clearly separated from the lecithin resonances, being 0.62 ppm downfield. The doublet character of each phospholipid resonance, due to the slightly different chemical shifts of the inside and outside phospholipids, is readily apparent. The addition of cobalt chloride completely broadens the outside resonances, leaving only the spectrum of the inside lecithin and sphingomyelin as is shown in fig. 3a. From these spectra it is calculated that the outside/inside ratio is 1.53 for lecithin and 1.97 for sphingomyelin. Since both DPL and sphingomyelin are neutral this result illustrates that the inside/outside distribution of phospholipid in mixed systems is as much a function of the packing properties of the individual lipids as of their charge [5].

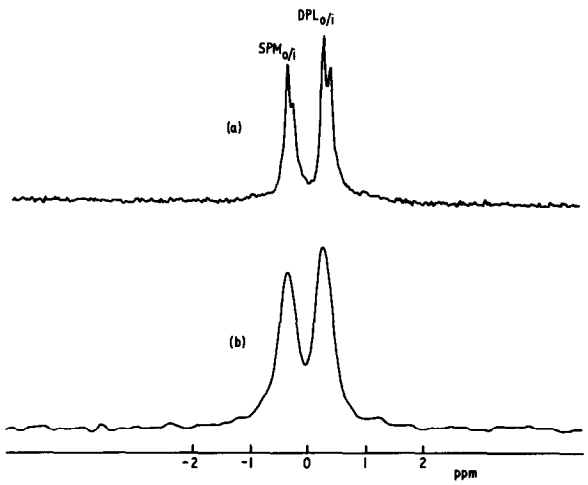

Fig. 2. ^{31}P NMR spectra of co-sonicated sphingomyelin (25 mg/ml) and dipalmitoyl lecithin (25 mg/ml) at 50°C: (a) 36 MHz; (b) 129 MHz.

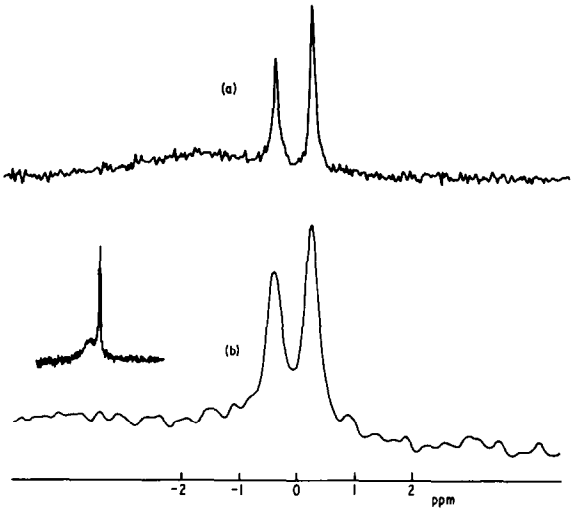

Fig. 3. ^{31}P NMR spectra of co-sonicated sphingomyelin (25 mg/ml) and dipalmitoyl lecithin (25 mg/ml) at 50°C in the presence of 2mM Co^{2+} in excess of EDTA: (a) 36 MHz; (b) 129 MHz. The insert in (b) shows the complete 129 MHz spectrum in the presence of Co^{2+} over a frequency width corresponding to 41.6 ppm.

Fig. 2b and 3b, respectively, show the ^{31}P NMR spectrum of the co-sonicated mixture of lecithin and sphingomyelin at 129 MHz and 50°C before and after the addition of Co^{2+}. While the lack of proton decoupling at 129 MHz introduces a small broadening (~5Hz), it is clear that the resolution is much reduced at the higher frequency.

Acknowledgements

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