^{31}P-NMR SIGNALS FROM INNER AND OUTER SURFACES OF PHOSPHOLIPID MEMBRANES

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1. Introduction

We showed earlier [1, 2] that PMR spectroscopy of phospholipid membranes in the presence of paramagnetic ions makes it possible to distinguish between signals coming from the inner and outer surfaces of a membrane, since the signal from the $N^+(CH_3)_3$ protons of the outer surface lecithin molecules is considerably shifted in comparison with the signal from the inner surface protons.

As there are grounds for believing that paramagnetic cations will interact with the negatively charged lecithin phosphate groups, one would expect larger chemical-shift differences in using ^{31}P-NMR spectroscopy.

2. Experimental

Egg lecithin was isolated by the procedure described elsewhere [1, 2]. For ^{31}P-NMR spectroscopy a sonicated 20% lecithin dispersion in D_2O [2] was used. The ^{31}P spectra were obtained on a Bruker BKR-323 high resolution Fourier transform NMR spectrometer operating at 36.436 MHz with proton noise decoupling and internal ^{2}D lock. The outside diameter of the spinning sample tube was 8 mm. The chemical shifts were referenced to external 85% H_3PO_4 recorded previous to sample measurement.

3. Results and discussion

Table 1 gives the results obtained for 20% sonicated lecithin dispersion in D_2O: *A*, before treatment with additives; *B*, on treatment with 0.01 M $Pr(NO_3)_3$; *C*, on subsequent treatment with 0.1 M KNO_3.

The ^{31}P-spectrum of the sample *A* is a singlet (fig. 1A). Subsequent addition of Pr^{3+} ions (sample *B*) results in two signals (fig. 1B), the signal in the higher field having practically the same shift as the sample *A* singlet. Clearly this upfield signal is due to the phosphate groups located on the inner membrane surface which do not come into contact with the Pr^{3+} ions, whereas the signal moving downfield belongs to the groups situated on the outer layer of the membrane.

The integral intensity ratio of the ^{31}P signals attributed to the inner and outer surfaces I_{out}/I_{in} is in accord with the PMR data [2] and satisfies the surface ratio for bilayer vesicles [3].

Treatment of sample *B* with KNO_3 leads to additional shifting of the low field ^{31}P signal. One could attribute this to stronger binding of Pr^{3+} to the membrane in the presence of KNO_3.

To compare ^{31}P-NMR and PMR data we calculated the reduced chemical shifts $\Delta\delta_{red}$ for equal concentrations of lecithin and Pr^{3+} as follows:

$$\Delta\delta_{red} = \frac{[Lec]\ (I_{out}/I_{in})}{[Pr^{3+}]\ (1+I_{out}/I_{in})}\ \Delta\delta$$

Table 1
^{31}P-NMR signals from outer and inner phosphate moieties of lecithin vesicles.

Sample	Inner surface		Outer surface		I_{out}/I_{in}
	Chemical shift δ (ppm)*	Line width $\Delta\nu_{1/2}$ (Hz)	Chemical shift δ (ppm)	Line width $\Delta\nu_{1/2}$ (Hz)	
A	1.10	50 ± 5	1.10	50 ± 5	–
B	1.37	67 ± 5	−11.52	116 ± 5	1.85
C	1.65	70 ± 5	−19.49	229 ± 5	1.98

* The small increase in δ-values of the internal ^{31}P signal observed for samples *B* and *C* must be due to alterations in macrosopic susceptibility of the sample induced by addition of paramagnetic ions.

Table 2
Pr^{3+}-induced shifts ($\Delta\delta_{red}$).

Sample	$\Delta\delta_{red}$ (ppm)		$\Delta\delta^{P}_{red}/\Delta\delta^{H}_{red}$
	$N^{+}(CH_3)_3$ [2]	^{31}P	
B	1.62	217.2	135
C	2.73	367.0	134

where I_{in} and I_{out} are integral intensities of the ^{1}H or ^{31}P signals from the inner and outer layer of the membrane respectively; $\Delta\delta$ is observed chemical shift at given concentrations of lecithin and Pr^{3+}.

Table 2 makes it clear that ^{31}P-NMR spectroscopy of lecithin vesicles is much more sensitive to the influence of paramagnetic ions that is PMR; it is also remarkable that the $\Delta\delta^{P}/\Delta\delta^{H}$ ratio does not change in the presence of diamagnetic KNO_3.

The intense shift of the ^{31}P signal under the action of hydrophilic paramagnetic probes, the simplicity of the ^{31}P spectra of phospholipids, and the possibility of assaying all phospholipids irrespective of whether or not they contain choline, makes ^{31}P-NMR spectroscopy competitive with PMR in investigation of artificial and biological membranes.

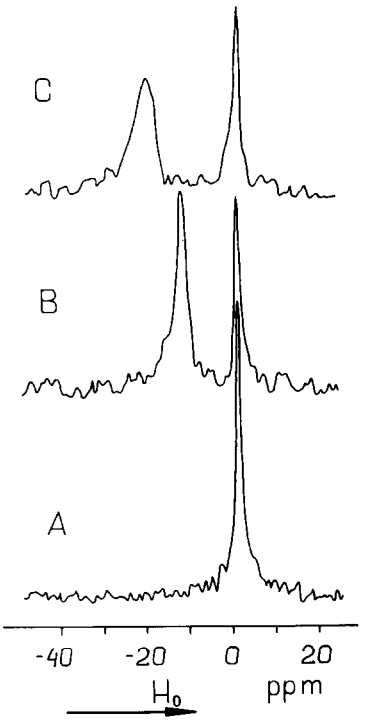

Fig. 1. ^{31}P spectra of a 20% (w/v) dispersion of egg yolk lecithin in D_2O (A); after addition to sample A of 0.01 M $Pr(NO_3)_3$ (B); after addition to sample B of 0.1 M KNO_3 (C).

References

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