

High-resolution ^{13}C NMR study of pressure effects on the main phase transition in L- α -dipalmitoyl phosphatidylcholine vesicles

(dynamics/model membranes/high pressure)

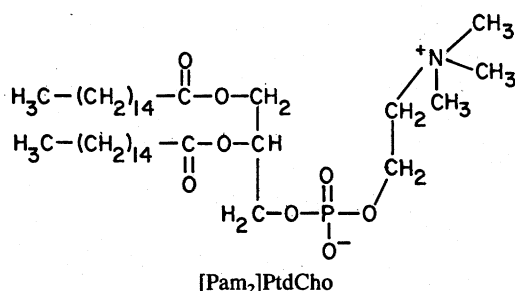
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Contributed by J. Jonas, February 19, 1988

ABSTRACT The effects of pressure on the liquid-crystalline to gel transition in vesicles of L- α -dipalmitoyl phosphatidylcholine were investigated by high-resolution proton-decoupled natural-abundance ^{13}C NMR spectroscopy. The linewidths of several ^{13}C resonances, including the choline methyl groups, carbonyl carbons, and choline methylene groups and the palmitoyl methyl groups are reported as a function of pressure at 52.7°C. These preliminary NMR experiments clearly demonstrate that high-pressure, high-resolution proton-decoupled natural-abundance ^{13}C NMR spectra are a promising tool to study the phase-transition behavior and the dynamics of model membrane systems.

It has been established (1-4) that high-pressure experiments can provide information about the dynamics and phase transitions in various biochemical systems. In particular, the high-pressure vibrational spectroscopic experiments by Wong (2) and the fluorescence measurements by Weber (3) on various phospholipid model membranes provided the motivation for the present study. Recently, we reported the value of high-pressure, high-resolution NMR spectroscopy in the investigation of the dynamic structure of liquids and developed a variety of probes to carry out those studies (5, 6). The great information content of high-resolution proton-decoupled natural-abundance ^{13}C NMR spectra of various model membrane systems has been well recognized (7) but, until the present study, all NMR experiments on such systems were performed only as a function of temperature, at atmospheric pressure. Therefore, we developed a high-resolution NMR probe to allow the measurement of proton-decoupled natural-abundance ^{13}C NMR spectra under high-pressure conditions. In this preliminary report, we describe experiments that deal with the effects of pressure on the liquid-crystalline to gel phase transition in L- α -dipalmitoyl phosphatidylcholine ([Pam₂]PtdCho) vesicles at 52.7°C. Our choice of [Pam₂]PtdCho follows from the fact that it is a well-studied model phospholipid for natural membrane systems and, specifically, it is found at high levels in lung surfactant.



The linewidths of several ^{13}C resonances were followed through the transition to prove the feasibility of high-resolution proton-decoupled ^{13}C NMR spectroscopy on biochemical systems at high pressure.

MATERIALS AND METHODS

[Pam₂]PtdCho (Sigma) was dispersed in glass-distilled water at a temperature above 41°C. The dispersion was sonicated with a Heat System/Ultrasonics (Plainview, NY) sonicator for a total time of 21 min, keeping the temperature of the samples close to 40°C. The final concentrations of the samples were 0.08-0.096 M.

The proton-decoupled natural-abundance ^{13}C NMR spectra were measured at 45.26 MHz using an NMR spectrometer system with quadrature detection, based on a wide-bore (130 mm) Oxford 4.2-Tesla superconducting magnet and a GE 1280 computer system. The high-resolution, high-pressure probe was a modification of a high-pressure NMR probe described earlier (8). The titanium high-pressure vessel has also been described earlier (6). A double-coil system was used in the NMR probe. Understandably, use of the specialized rf transmission lines from the high-pressure environment to ambient pressure in the probe plug with the tuning capacitors caused problems not usually encountered when designing a high-resolution NMR probe for work at atmospheric pressure. The temperature was measured using a copper-constantan thermocouple inside the high-pressure vessel, and the temperature was controlled by circulating water with a Brinkman thermostat circulator, model NB, through a thermostating jacket around the high-pressure vessel. The temperature was estimated to be accurate to within $\pm 0.5^\circ\text{C}$.

The most important performance features for the high-pressure, high-resolution NMR probe are sensitivity and resolution. Sensitivity is defined as the ^{13}C signal-to-noise ratio using a 90° pulse and a nonspinning sample. The sensitivity for our high-pressure probe with an 8-mm nonspinning sample is 60 with a matched exponential filter (9), which reaches half the value achievable on a commercial 200-MHz NMR spectrometer (10). The resolution for the 8-mm nonspinning samples is exceptional: 2 Hz for protons (resonant frequency, 180 MHz) and 0.5 Hz for ^{13}C .

The experimental conditions were as follows: the sweep width was 6 kHz with CW proton decoupling, 7000-12,000 scans were accumulated, and a 3-Hz line broadening was used in data treatment to improve the signal-to-noise ratio.

RESULTS AND DISCUSSION

The reason why we chose to follow the main liquid-crystalline to gel phase transition in [Pam₂]PtdCho by monitoring the linewidth of the various natural-abundance ^{13}C

Abbreviation: [Pam₂]PtdCho, dipalmitoyl phosphatidylcholine.

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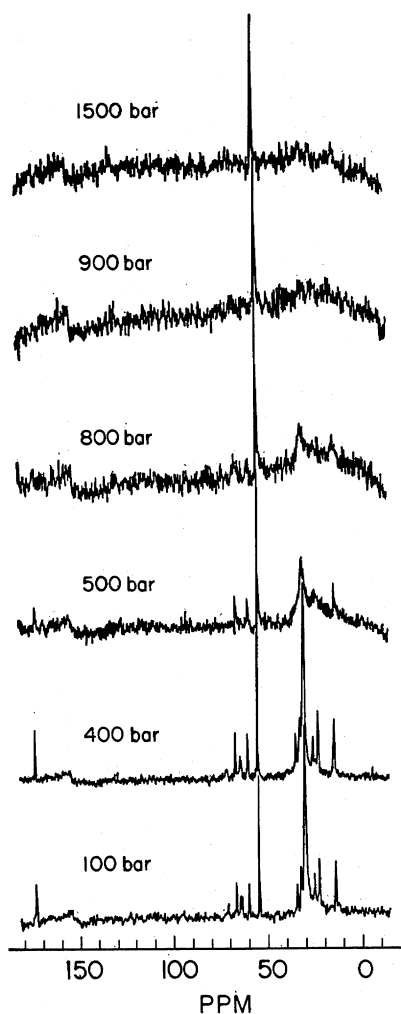


FIG. 1. Proton-decoupled natural-abundance ^{13}C Fourier-transform spectra of $[\text{Pam}_2]\text{PtdCho}$ in H_2O as a function of pressure at 52.7°C .

resonances is evident when we consider the expressions (11) for the spin-lattice relaxation time (T_1) and the spin-spin relaxation time (T_2) given below:

$$1/T_1 \propto A[J_1(\omega_0) + J_2(2\omega_0)], \quad [1]$$

where $J_1(\omega_0)$ is the Fourier transform of the correlation function at the resonance frequency ω_0 and A is a constant related to internuclear separation. The relaxation rate $1/T_1$

Table 1. Assignment of ^{13}C resonances in $[\text{Pam}_2]\text{PtdCho}$

Resonance	Chemical shift, ppm
Palmitoyl methyl	16
First methylene	25
Second methylene	28
Bulk methylene	31
Methylene	34, 36
Choline methyl	56
Choline methylene	61
Glycerol methylene	65, 65.5
Choline methylene	68
Glycerol methylene	73
Carbonyl	175

Resonances are assigned according to ref. 10. Chemical shifts are referenced to tetramethylsilane.

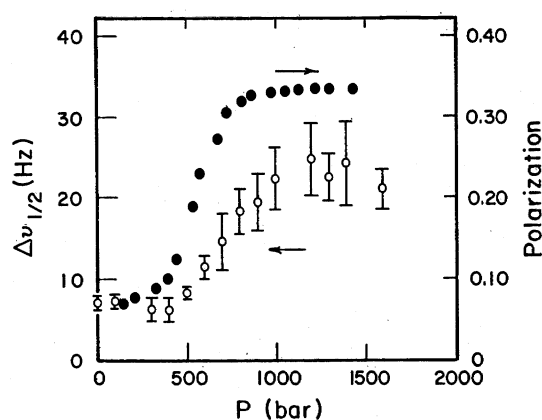


FIG. 2. Comparison between the pressure dependence (\circ) of the ^{13}C linewidth at half-maximum ($\Delta\nu_{1/2}$) for the choline methyl groups in $[\text{Pam}_2]\text{PtdCho}$ vesicles at 52.7°C and the fluorescence data (\bullet) reported by Chong and Weber (13). The fluorescence data have been corrected for the temperature difference. Pressure dependence results represent mean \pm SEM.

reflects motions at ω_0 and $2\omega_0$. In contrast, the expression for T_2 shows that $1/T_2$ monitors slow motions

$$1/T_2 \propto B[J_0(0) + J_1(\omega_0) + J_2(2\omega_0)], \quad [2]$$

where $J_0(0)$ is the Fourier component of the correlation function at zero frequency. Since the linewidth $\Delta\nu_{1/2}$ (full-width at half-maximum intensity) is proportional to $1/T_2$, the changes of linewidth will reflect changes in the mobility of various carbons in the $[\text{Pam}_2]\text{PtdCho}$ molecule.

The proton-decoupled natural-abundance ^{13}C Fourier-transform NMR spectra of $[\text{Pam}_2]\text{PtdCho}$ at 52.7°C as a function of pressure is shown in Fig. 1. These high-resolution spectra demonstrate the feasibility of using high-pressure, high-resolution proton-decoupled natural-abundance ^{13}C NMR spectroscopy to provide information about a phase transition and the dynamics in a model membrane. The assignment (see, e.g., ref. 12) of the main ^{13}C resonances is given in Table 1. As the pressure increases, one moves from a bilayer in which the palmitoyl chains are very flexible and mobile into the first gel state, in which the bilayer organization is retained but the acyl chains become rigid. The choline head group maintains considerable mobility, even in the gel

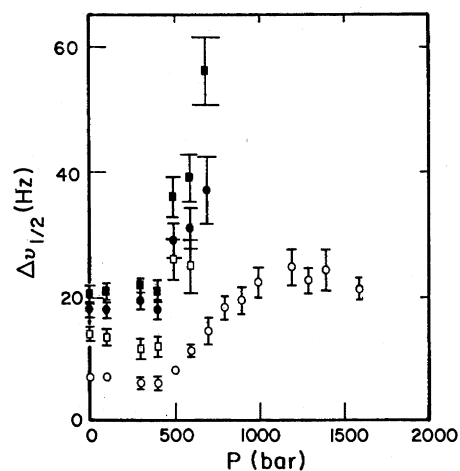


FIG. 3. Pressure dependence of ^{13}C linewidths in $[\text{Pam}_2]\text{PtdCho}$ vesicles for choline methyl groups (\circ), choline methylene groups (\square), palmitoyl methyl groups (\bullet), and carbonyl carbons (\blacksquare) at 52.7°C . Results represent mean \pm SEM.

phase, because it is exposed to water, and its methyl groups can rotate quite freely even in a very hindered environment.

The pressure dependence of the ^{13}C linewidth of the choline methyl groups [Pam₂]PtdCho is shown in Fig. 2. The increase of linewidth accompanying the main liquid-crystalline-gel transition is as expected, with the relatively high mobility of the choline methyl groups also evident in the gel phase. It is of interest to compare the liquid-crystalline to gel transition induced by pressure and detected by using the fluorescent probe diphenylhexatriene (13) with the results of the present NMR study. The diphenylhexatriene is a hydrocarbon that is located in the interior of the bilayer and reflects the motions and organization of the acyl chains but is not sensitive to the motions of the choline methyl groups. The data points were shifted to the right by 132 bars (1 bar = 100 kPa) to compensate for the temperature difference. Even if one corrects for the difference in the temperature between the two experiments, the data indicate that the choline methyl groups have a higher phase-transition pressure than the hydrocarbon domains of the bilayer.

The linewidth results shown in Fig. 3 indicate that, in the liquid-crystalline state, the carbonyl carbons in the glycerol backbone region have the most restricted motions, the acyl chain methyl groups have intermediate mobility, and the methylene and methyl carbons of the head group are most mobile. The same relative differences of mobility are observed as the [Pam₂]PtdCho bilayer enters into the phase transition, but the phase-transition pressures for the carbons in the acyl chains appear to be lower than that for the choline methyl groups. By 800 bars, the motions of most carbon atoms in [Pam₂]PtdCho become so hindered that their resonance peaks broaden beyond detection; the choline methyl carbons, however, remain sufficiently mobile in the gel state to give a sharp resonance peak. Furthermore, we found that the effects of pressure on the ^{13}C NMR spectra of [Pam₂]-

PtdCho are reversible; therefore, the line broadening is due to the phase transition and not to an irreversible vesicle aggregation or fusion.

In summary, we have shown that it is possible to carry out high-pressure, high-resolution natural-abundance ^{13}C NMR experiments and to obtain information about the phase-transition behavior and dynamics of model membrane systems.

Our thanks are due to C. Reiner for his help with the probe design. This work was supported in part by National Science Foundation Grant CHE 85-09870 and National Institutes of Health Grant PHS-HL-16059.

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