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PHASE TRANSITIONS OF PHOSPHATIDYLCHOLINE BILAYERS AS REVEALED BY CROSS-POLARIZATION EFFICIENCY OF ^{31}P-NMR

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Cross-polarization efficiency is found to be an excellent NMR parameter to reflect dynamic properties of phospholipid bilayers. This parameter is quite sensitive to the pretransition rather than the main transition, in contrast to other NMR parameters studied so far. An investigation of the cross-polarization efficiency provides us with information on the relatively slower motions of the phospholipid molecules.

The dynamic state of phospholipid bilayers is very important in connection with biological functions of biomembranes. A dramatic change in the dynamic state takes place through the phase transition of the lipid bilayers. Nuclear magnetic resonance (NMR) is one of the most powerful methods which can offer information on the dynamics. Extensive studies on the lipid bilayers by NMR have been carried out in the last decade [1–4]. All NMR parameters studied so far respond well to the main transition but are not so sensitive to the pretransition [1–4]. We would like to present an NMR method which is more sensitive to the phase behavior and gives information about slower motions of the phospholipid molecules in bilayers.

^{31}P-NMR spectra of the multilamellar liposomes of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) at 40.3 MHz are shown in Fig. 1. DPPC was purchased from Sigma Chemical Co. Purity was checked by thin-layer silica gel chromatography. About 60 mg of DPPC was dispersed at 50 °C in a tube containing 0.5 ml of 1 mM EDTA/10 mM Tris buffer (pH 7.3). The tube was put in an NMR tube for the measurements after centrifugation to pack the sample. The spectra were recorded on a JEOL FX-100 solid-state NMR system. A high-power probe head with a specially designed variable temperature control was used. The spectra were obtained by using either a single-pulse or a cross-polarization pulse sequence [5]. High-power proton decoupling was carried out during the aquisition of a free-induction decay in both cases. Line broadening of 100 Hz was applied in each spectrum.

As is well known, the spectra show powder patterns typical for the phosphorus undergoing axially symmetric motions, suggesting that the motional correlation time of the phosphate group parallel to the bilayer-normal ($\tau_{\parallel}$) is much shorter than 10^{-5} s. The most significant feature of Fig. 1 is a change in the intensity of the spectrum obtained by the cross-polarization method. The integrated intensity of the spectrum is plotted as a function of temperature in Fig. 2. Fairly large changes can be seen at about 33 °C and 41 °C. These temperatures correspond to the pre- and main transitions of the DPPC bilayer, respectively [6]. The intensity of the spectrum obtained by single pulse is also plotted in Fig. 2.

In contrast to the former case, the temperature dependence is very small and constant. Since the tuning of the probe head scarcely depended on

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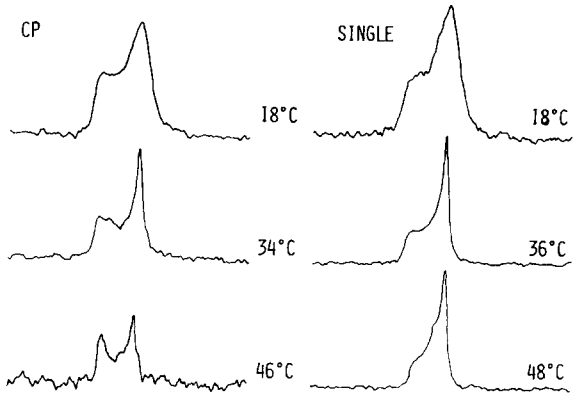

Fig. 1. ^{31}P-NMR spectra of DPPC bilayers at 40.3 MHz and at various temperatures. CP: measured by the cross-polarization pulse sequence with 11.3 G proton-locking field, 3 ms mixing time and 3 s pulse delay. The Hartmann-Hahn condition was matched at 18°C. Single: measured by a single pulse ($\pi/4$ pulse) with 3 s pulse delay.

temperature, the large intensity changes should be associated with the cross-polarization efficiency. Thus, it can be concluded that both of pre- and main phase transitions induce remarkable changes in the cross-polarization efficiency. Furthermore, the lineshape of the cross-polarization spectra at 34 and 46°C in Fig. 1 suggest that the cross-polarization efficiency depends on the orientation of the director axis of the molecule. Since NMR studies have so far failed to show any significant change at the pretransition of the phospholipid bilayer, the cross-polarization efficiency appears to be an excellent parameter for the investigation of dynamic properties of the phospholipid bilayers.

The cross-polarization efficiency is dominated by two time constants, namely the cross relaxation time between proton and phosphorus spins (T_{HP}) and the spin-lattice relaxation time of protons in the rotating frame ($T_{1\rho}$) [5]. When T_{HP} becomes shorter or $T_{1\rho}$ longer, the efficiency rises. Since cross-polarization is mediated by the dipole-dipole interactions, T_{HP} is sensitive to the motions which average out the dipole-dipole interactions. The correlation time of such motion is shorter than 10^{-5} s. The correlation time which gives the minimum of $T_{1\rho}$ is expected to be $2 \cdot 10^{-6}$ s under our experimental conditions. Therefore, the change in the cross-polarization efficiency represents a change in such slow motions that have correlation times in the range of 10^{-7}–10^{-4} s. The significant changes at the pre- and main transitions in Fig. 2 clearly show that remarkable changes in the slow motions mentioned above occur in concomitant with these phase transitions. A quantitative analysis of the cross-polarization efficiency of this system will be given elsewhere.

In the cross-polarization experiments, the Hartmann-Hahn conditions $\gamma_H H_{1H} = \gamma_P H_{1P}$ should be matched, where γ and H_1 are the gyromagnetic ratio of a nucleus and the magnetic field of a radio wave, respectively. The Hartmann-Hahn condition was determined at 18°C for the spectra in Fig 1. When the condition was matched at 52°C, a different temperature profile was obtained. The integrated intensity of the spectrum is plotted as a function of temperature in Fig. 3. Although the change at the pretransition is similar to that in Fig. 2, the change at the main transition is diminished. This fact suggests that the Hartmann-Hahn condition is not satisfied in the liquid-crystalline state in Fig. 2, while the condition is satisfied in the gel and intermediate states in both Figs. 2 and 3. This can be explained as follows. Since the dipole-dipole interactions are very strong in the gel and intermediate states, the Zeeman energy levels widely distribute. In other words, the resonance line without decoupling has a very broad linewidth. As a result, either H_{1H} or H_{1P} can satisfy the Hartmann-Hahn condition over a wide range.

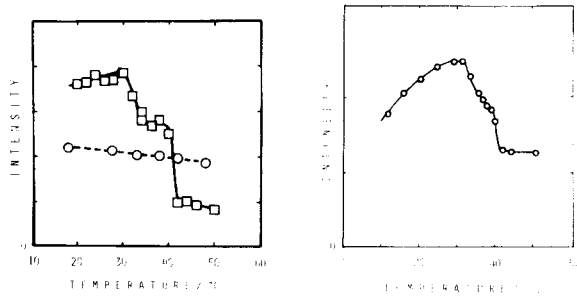

Fig. 2. Temperature-dependence of the integrated intensity of ^{31}P-NMR spectrum of DPPC bilayers, □, by cross-polarization; ○, by single pulse.

Fig. 3. Temperature dependence of the integrated intensity of ^{31}P-NMR spectrum of DPPC bilayers observed by cross-polarization. The Hartmann-Hahn condition was matched at 52°C. Other conditions are the same as those given in Fig. 1.

By contrast, the distribution of the Zeeman energy levels becomes narrower in the liquid-crystalline state because of the averaging of the dipole-dipole interactions by enhanced molecular motions. Thus the range to satisfy the Hartmann-Hahn condition becomes narrower in the liquid-crystalline state. Using the difference in this range, one can exaggerate the change in the cross-polarization efficiency at the main transition as shown in Fig. 2.

As can be seen in the temperature profile of Fig. 3, the retained intensity in the liquid-crystalline state is still relatively strong. This fact indicates, that despite the averaging of the dipole-dipole interactions, the nonvanishing interactions are still efficient enough to mediate the cross-polarization.

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