CHEMISTRY LETTERS, pp. 717-718, 1985.

^{14}N NUCLEAR QUADRUPOLE SPLITTINGS OF PHOSPHATIDYLCHOLINE IN BILAYERS. THE INFLUENCE OF NEGATIVE CHARGE IN THE HEAD GROUPS

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The ^{14}N nuclear quadrupole splitting of phosphatidylcholine was measured in the presence of acidic lipids in multibilayers. The splitting was found to increase with negative charge in mixed lipid systems.

The usefulness of ^{14}N NMR has been demonstrated for the study of head group interactions of phosphatidylcholine(PC) in bilayers.[1-3] The ^{14}N nuclear quadrupole splitting $\Delta\nu_Q(^{14}N)$ of PC in various multibilayers has been shown to be sensitive to the addition of other substances such as cholesterol,[4)] proteins,[4,5)] metal ions, and anesthetics.[4,6)] The magnitude of $\Delta\nu_Q(^{14}N)$ tends to decrease by such perturbations, but general interpretation has not been given to the mechanism of $\Delta\nu_Q(^{14}N)$ change by those substances. In this work, we studied the effect of acidic lipids on $\Delta\nu_Q(^{14}N)$ with an attempt of finding a mechanism of $\Delta\nu_Q(^{14}N)$ change caused by foreign substances and of developing a new method of detecting the molecular perturbations at the surface of lipid bilayers using ^{14}N NMR.

Phosphatidylcholine was extracted from hen egg, phosphatidylserine(PS) was extracted from bovine brain white matter, and phosphatidic acid(PA) was prepared from PC with phospholipase D from cabbage. The multibilayer dispersions(150 g/dm^3) were prepared in a maleate buffer of 10 or 20 mM(1 M=1 mol dm^{-3}) with 0.1 or 1 mM ethylenediaminetetraacetic acid and 0.1 M NaCl. The mole fraction of PS, X_{PS}, in the final dispersion was determined by a combination of the quantitative analyses of phosphorus and primary amine and of PA, X_{PA}, by ^1H NMR signals of $-N^+(CH_3)_3$ and $-CH_3$. Ordinary FT spectra of ^{14}N were obtained by JEOL GX-400 spectrometer at 28.9 MHz with a 45° pulse of 20 - 30 μs at the spectral width of 100 kHz. Temperature was checked by a thermocouple in the NMR tube containing the buffer (± 0.2 °C).

The ^{14}N NMR spectrum of PC in multibilayer dispersion(Fig.1) showed a powder pattern[7)] except that the 0° edges were not clearly exhibited due to a wide pulse width and the instrumental dead time(180 μs) before the data aquisition. The line shape was not seriously affected by the acidic lipid incorporation up to X_{PS} or X_{PA} of 0.4. The ^{14}N quadrupole splitting $\Delta\nu_Q(^{14}N) = (3/4)(e^2qQ/h)S_{C_\beta-N}$ was obtained from the sharp 90° edges, where e^2qQ/h is the principal component of the axially symmetric ^{14}N nuclear quadrupole coupling tensor of $\underset{\alpha}{C}H_2\underset{\beta}{C}H_2N^+(\underset{\gamma}{C}H_3)_3$ and $S_{C_\beta-N}$ is the order parameter of the axis with respect to the bilayer normal. In contrast to the other perturbations,[4-6)] acidic lipids gave an increase in $\Delta\nu_Q(^{14}N)$. It increased with X_{PS} or X_{PA} and in the order PS(pH 3.9) $\lesssim$ PS(pH 7) $\lesssim$ PA(pH 7) at constant

X(Fig. 2A,B). From these results and the pK of lipids, $pK_2(PS) \simeq 4$, $pK_3(PS) \simeq 10$, and $pK_2(PA) \simeq 8.5$, it is suggested that the electric charge in the head group is the dominant factor determining the value of $\Delta\nu_Q(^{14}N)$ rather than the bulkiness of the head group.

A conformational change of head group has been suggested to cause the complicated behaviors of deuteron quadrupole splittings $\Delta\nu_Q(^2H)$ at α, β, and γ positions of PC due to the introduction of acidic lipids such as PS.[8] However, it is not simple to give an interpretation simultaneously applicable to the increase in $\Delta\nu_Q(^{14}N)$ and the decrease in $\Delta\nu_Q(^2H)$ at β and γ positions due to PS addition by conformational effect alone. A change in the value of e^2qQ/h might participate in this case under a strong electrostatic field generated by the excess charge in the membrane[8] although no e^2qQ/h change has been detected in the case of small trimethylalkylammonium ion in solution of wide range of pH and temperature.[9] An extensive study including $T_1(^{14}N)$ measurements and the incorporation of positively charged amphiphiles is in progress.

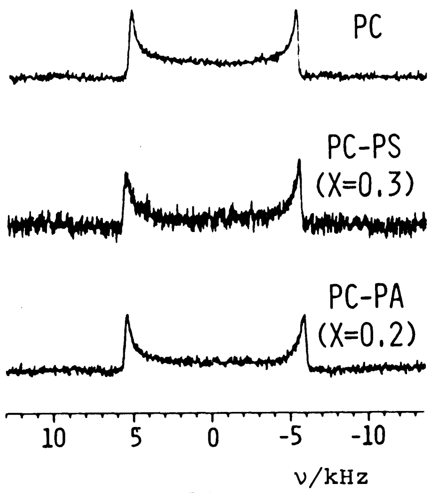

Fig. 1. ^{14}N NMR spectra of PC in various multibilayers(30 °C, pH 7).

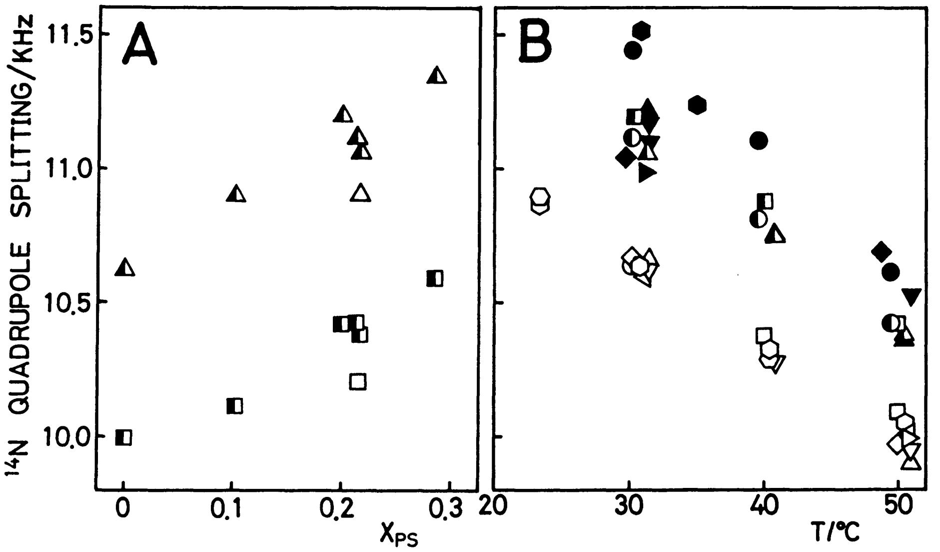

Fig. 2. ^{14}N quadrupole splittings of PC. (A) $\Delta\nu_Q$ vs. X_{PS}. pH 7(half closed), pH 3.9(open), 30 °C(triangle), and 50 °C (square). (B) $\Delta\nu_Q$ vs. temperature at pH 7. PC(open), PC-PS(X_{PS}=0.2, half closed), and PC-PA(X_{PA}=0.2, closed). Symbols of different shapes(B) indicate different samples.

This work was supported by the Joint Studies Program(1983 - 1984) of the Institute for Molecular Science. We thank Professor Hidemasa Takaya of the institute for his invaluable supports to the NMR measurements on GX-400.

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(Received February 28, 1985)