Vol. 95, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1980 Pages 269-272

THE LATERAL DIFFUSION COEFFICIENT OF LECITHIN MOLECULES IN SINGLE BILAYER VESICLES STUDIED BY ^{14}N NMR

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Received May 21,1980

SUMMARY

The ^{14}N nuclear relaxation times T_1 and T_2 in egg yolk phosphatidylcholine have been observed in single bilayer vesicles dispersed in the media of different viscosities, ^1H_2O and ^2H_2O. The lateral diffusion coefficient of lipid molecule D has been calculated according to the method reported earlier: D = 2.2 x $10^{-8}cm^2s^{-1}$ in ^1H_2O and 2.1 x $10^{-8}cm^2s^{-1}$ in ^2H_2O at 20°C. They are in excellent agreement. This result gives a strong basis of usefulness of ^{14}N NMR method in the evaluation of D without introducing any system perturbation.

INTRODUCTION

We have shown that ^{14}N NMR is potentially a powerful tool for the study of the dynamical properties of molecules in single bilayer vesicles such as lateral diffusion coefficient and correlation time for the local motion without any system perturbation (1). The quadrupole relaxation times T_1 and T_2 of ^{14}N nucleus are determined by the motions of the principal axes of the electric field gradient tensor associated with the nucleus. The effective motions for the nuclear relaxation in the case of lecithins in single bilayer vesicles, which reorient the director of N-C_β bond [··P-O-CH_2-CH_2-$N(CH_3)_3$], consists of the local motion (molecular rotation and segmental motion), vesicular tumbling motion, and the lateral diffusion of the molecule.

In the present study using a deuterium isotope effect on the solvent viscosity, it was confirmed that the method of ^{14}N relaxation was a reliable tool for the evaluation of lateral diffusion coefficient.

0006-291X/80/130269-04$01.00/0

Table I. ^{14}N T_1 and line width of lecithin in single bilayer vesicles dispersed in light and heavy water.

TEMP. /°C	^1H_2O		^2H_2O	
	T_1/ms	$\Delta\nu_{1/2}$/Hz	T_1/ms	$\Delta\nu_{1/2}$/Hz
20	27.2 ± 1.1	206.4 ± 6	24.7 ± 0.7	243.0 ± 10
35	(52.9)	120.0 ± 1	43.8 ± 0.4	133.0 ± 8
50	90.4 ± 3.8	73.8 ± 3	76.8 ± 0.4	78.3 ± 4

All the data at 20°C and the width in ^1H_2O at 50°C were the averages of 5 to 7 observations and the others were the averages of 2 to 3 observations except for T_1 in parenthesis which was the interpolated value from the data taken at 5 different temperatures. The standard deviations were used as the error estimates.

EXPERIMENTAL

The single bilayer vesicles of egg yolk phosphatidylcholine (Singleton method) were prepared by ultrasonic method in the buffer solutions containing 0.1 M NaCl, 2 mM N-tris-(hydroxymethyl)-methyl-2-aminoethane sulfonic acid, 2mM L-histidine, and 0.1 mM ethylenediaminetetraacetic acid in ^1H_2O(pH 7.4 and 6.8) and in ^2H_2O(99% in deuterium, pD 7.4: a direct reading of pH meter). ^{14}N NMR was taken at 7.2 MHz by the FT NMR system reported previously (2). The spin lattice relaxation time T_1 was obtained by the inversion recovery method and T_2 from the half full width $\Delta\nu_{1/2}$.

RESULTS

No pH dependence was observed in the nuclear relaxation data taken at pH 7.4 and 6.8 in ^1H_2O. T_1 and $\Delta\nu_{1/2}$ measured at pH 7.4 in ^1H_2O and pD 7.4 in ^2H_2O are listed in Table I.

The quadrupole relaxation times of ^{14}N nucleus in single bilayer vesicles can be expressed by the following relations (1).

$$1/T_1 = (3\pi^2/10)(e^2qQ/h)^2[\{\tau_R/(1+\omega_0^2\tau_R^2) + 4\tau_R/(1+4\omega_0^2\tau_R^2)\}S^2 + 5\tau_r f(S)]$$

$$1/T_2 = \pi\cdot\Delta\nu_{1/2} = (3\pi^2/10)(e^2qQ/h)^2[\{3\tau_R/2 + 5\tau_R/\{2(1+\omega_0^2\tau_R^2)\} + \tau_R/(1+4\omega_0^2\tau_R^2)\}S^2 + 5\tau_r f(S)]$$

where τ_r is the reorientational correlation time for the N-C_β

bond director due to the local motion and τ_R is the one due to the motion along the surface of the vesicle

$$1/\tau_R = 1/\tau_a + 1/\tau_d = 3kT/4\pi a^3\eta + \langle 6D/R^2 \rangle$$

consisting of the contributions from the vesicle tumbling motion τ_a which can be evaluated according to the Stokes-Einstein relation and from the lateral diffusion of molecules τ_d on the surface of the sphere with radius R where the average $\langle 6D/R^2 \rangle$ is taken for inner and outer surfaces of the vesicle. S is the order parameter of N-C_β director with respect to the normal of vesicle surface, and $f(S)$ is approximated by 1 when S is small compared with 1 (3). The other simbols have their usual meanings.

The lateral diffusion coefficients D's in ^1H_2O and in ^2H_2O could be calculated independently by setting the common values a and S under the assumption that only the viscosity η is influenced by the solvent isotope effect. The results at 20°C were $D(^1H_2O) = 2.1_6 \pm 0.4 \times 10^{-8} cm^2 s^{-1}$ and $D(^2H_2O) = 2.1_5 \pm 0.5 \times 10^{-8} cm^2 s^{-1}$ with the vesicle radius a = 105.6 A (4), S = 0.106 (1), $3e^2qQ/4h$ = 83.5 kHz (5), and $\eta(^1H_2O$, 0.1 M NaCl) = 1.01 cp and $\eta(^2H_2O$, 99%-d, 0.1 M NaCl) = 1.26 cp. The agreement between these diffusion coefficients is excellent.

The correlation times for the local motion τ_r observed in ^1H_2O and ^2H_2O are listed below. The solvent isotope effect on τ_r was beyond the experimental error of 4%.

	20°C	35°C	50°C
$\tau_r/10^{-10}$s in ^1H_2O	2.0	1.0_1	0.59
$\tau_r/10^{-10}$s in ^2H_2O	2.2	1.2_3	0.70

DISCUSSION

The lateral diffusion coefficients of lecithin molecules in single bilayer vesicles obtained independently in ^1H_2O and in

^2H_2O were in good agreement. In addition, the value of D agrees well with those obtained by the other techniques such as fluorescent probe method, spin probe ESR, and proton relaxation. The adequacy of the method of ^{14}N relaxation for the evaluation of the diffusion coefficient is thus shown.

There was no detectable change in the ^{14}N relaxation data when pH was changed from 7.4 to 6.8 at 20°C in ^1H_2O. The motional properties of neutral lipid seem to be insensitive to pH near the neutral region. The longer τ_r's in ^2H_2O than in ^1H_2O, which indicated a more restricted motion of the lecithin head group in ^2H_2O, must be caused by the difference in the hydrogen bonding abilities of these solvents. Lecithin polar region has hydrogen bonds with surrounding water molecules which is stronger with ^2H_2O than with ^1H_2O.

Since the solvent isotope effect on the local dynamical properties is shown, the effect on the order parameter S is also expected. For a more detailed discussion on the evaluation of diffusion coefficient, therefore, the measurement of ^{14}N quadrupole splitting $\Delta\nu_Q = (3/4)(e^2qQ/h)S$ will be required.

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