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Dynamical Structure of Phosphatidylcholine Molecules in Single Bilayer Vesicles Observed by Nitrogen-14 Nuclear Magnetic Resonance†

Keiko Koga and Yoko Kanazawa*

ABSTRACT: A ^{14}N nuclear magnetic resonance (NMR) study of lecithins, 24–65 °C for dipalmitoylphosphatidylcholine (DPPC) and 20–50 °C for egg yolk phosphatidylcholine (EPC), in single bilayer vesicles prepared by an ultrasonic method is reported. Choline ^{14}N signals are found to be Lorentzian in these systems of definite molecular arrangement because of the smallness of the quadrupole coupling constant. This is due to the high symmetry around the trimethylammonium type nucleus and to the low ordering compared with thermotropic liquid crystals. An analysis of relaxation times T_1 and T_2 gives the following results. (1) The activation energies for the rapid local motion of C_β–N bonds are 36 and 31 kJ·mol^{-1} for DPPC and EPC, respectively. These are higher than that of a C_α–D bond, indicating that the $-N^+(CH_3)_3$ group is bound in the polar surface. (2) The vesicle radius seems to decrease toward higher temperatures, just below the start of the phase transition. (3) The lateral diffusion coefficient of the constituent lipid is obtained with the help of the Stokes radius. The ^{14}N NMR technique has the essential advantage of applicability without modification of the system.

The physical properties of model membrane systems consisting mainly of phospholipids have been investigated by various methods. The significance of spectroscopic work is evident for the dynamical study of membrane phenomena at the molecular level. *N*-Oxide spin-label electron spin resonance (ESR)[1] has been a powerful technique to detect various physical properties (see, e.g., Wu & McConnell, 1975; Ito & Ohnishi, 1975). The fluorescent probe method has also been used extensively to observe the fluidity and phase transitions of the membranes (see, e.g., Lentz et al., 1976; Wu et al., 1977). These techniques necessarily include the use of probes, which are either modified lipids or foreign molecules, giving more or less modification of the system.

For studies of the hydrophilic part of the membrane and of perturbations of the system such as the effects of drugs, the

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[1] Abbreviations used: ESR, electron spin resonance; NMR, nuclear magnetic resonance; FT, Fourier transform; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; EPC, egg yolk phosphatidylcholine; CTAB, *n*-hexadecyltrimethylammonium bromide; HTAB, *n*-hexyltrimethylammonium bromide.

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observation of the original constituent lipid is essential. Here, nuclear magnetic resonance (NMR) is a very powerful tool. Many studies on ^{1}H, ^{2}H (D), ^{13}C, and ^{31}P have been reported in this field (see, e.g., de Kruijff et al., 1975; Gally et al., 1975; Stockton et al., 1976; Gent & Prestegard, 1977; Bloom et al., 1978). Important information is obtained by ^{1}H NMR, but data analysis is difficult because of the coexistence of many nonequivalent proton nuclei. The exact treatment of ^{13}C and ^{31}P NMR requires a separation of several relaxation mechanisms such as dipolar interaction, spin–rotation interaction, and chemical-shift anisotropy. A more convenient tool for the study of ordered molecules has been ^{2}H NMR, where the dominant mechanism of nuclear relaxation is the coupling of nuclear quadrupole moment with the electric field gradient at the nucleus, modulated by the molecular motion. The correlation time for the local molecular or segmental motion has been obtained from the longitudinal relaxation time T_1 and that for the motion associated with the whole aggregate has been obtained from the transverse relaxation time T_2 when these motions are sufficiently fast. The order parameter S of the C–D bond director in the lamellar phase has been obtained from the quadrupole splitting. One serious disadvantage of the ^{2}H NMR technique is the necessity of isotopic labeling.

Recently, a study of ^{14}N relaxation times, T_1 and T_2, of *n*-hexadecyltrimethylammonium bromide (CTAB) and chloride in micelles has been reported by Henriksson et al. (1977), giving a good figure for the micelle shape of these systems. The advantage of the ^{14}N nucleus as an NMR probe is in its isotopic abundance and the simple mechanism of nuclear relaxation. Moreover, the nitrogen nucleus, which exists mostly as the positively charged state in the membrane lipid, is expected to play an important role in intermolecular interaction. It is our purpose to investigate the applicability of ^{14}N NMR as a technique for a motional analysis and for the direct observation of electrostatic interaction sites in the model membrane without any molecular or system modification.

In the present work, the ^{14}N NMR spectra and relaxation times of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) and egg yolk phosphatidylcholine (EPC) were measured in single bilayer vesicles and analyzed for the first time. T_1 is determined mainly by the rapid motion of the C_β–N bond and was used for the evaluation of local molecular or segmental motion. The line shape was found to be Lorentzian and T_2 could be obtained from its width. It was demonstrated that the lateral diffusion coefficient of lipid molecules in the vesicle could be evaluated without system modification from the relaxation rates.

Materials and Methods

Reagent grade CTAB was purchased from Merck (West Germany), tetramethylammonium bromide was from Nakarai Chemicals (Japan, for polarography), and reagent grade ammonium chloride was from Ishizu Pharmaceutical (Japan). The chromatogram of DPPC purchased from Fluka (Switzerland, lots 195536-117 and 199699-478) in the solvent chloroform/methanol/water (65:25:4 v/v/v) on silica gel (Wako Pure Chemical Industries, Japan) was a single spot. EPC was extracted from egg yolks and purified by aluminum oxide (Woelm, West Germany) column chromatography according to the method of Singleton et al. (1965).

The dispersion of multilamellar liposomes was prepared following the method of Bangham et al. (1965) in a pH 7.4 buffer solution containing 0.1 M NaCl, 2 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, 2 mM L-histidine, and 0.1 mM ethylenediaminetetraacetic acid. The dispersion was concentrated by centrifugation at 3000 rpm for 10 min, followed by dilution to 0.2 M, and was sonicated with a homemade bath-type ultrasonicator at 40 kHz. Sonication was performed under an Ar atmosphere at 50 °C in the case of DPPC and at ~20 °C in EPC for about 1 h until the liquid became optically clear. The size of sonicated vesicles was checked by gel chromatography on Sepharose 4B (Pharmacia Fine Chemicals, Sweden).

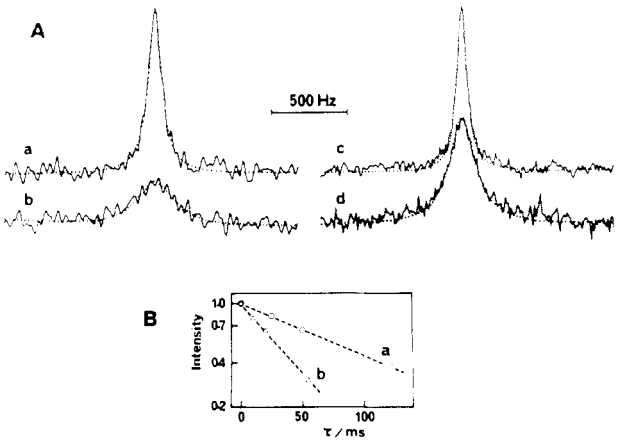

FIGURE 1: ^{14}N NMR spectra of lecithins in single bilayer vesicles at 7.2 MHz. (A) FT spectra obtained with 10 Hz/point in the frequency domain and 2048 data points (—) and the Lorentzian fittings (···). Integral intensities of the spectra were constant within 10% after correction for the dead time before signal sampling: (a) DPPC at 50 °C, 16 384 transients, Y gain 1; (b) DPPC at 35 °C, 32 768 transients, the base line of Lorentzian was corrected, Y gain 2; (c) EPC at 50 °C, 16 384 transients, Y gain 1; (d) EPC at 20 °C, 32 768 transients, Y gain 2. (B) Inversion recovery process plotted vs. pulse interval τ: (a) DPPC at 60 °C, 4096 transients; (b) DPPC at 41 °C, 8192 transients.

^{14}N NMR spectra were obtained at 7.2 MHz by a JEOL-PS-100 electromagnet and a homemade pulse Fourier transform (FT) system (Kanazawa et al., 1979). The temperature was controlled by a JNM-VT-3T unit. Its reliability was monitored by the alternative insertion of a thermometer to the sample position, where the temperature was ±0.5 °C. Spectra were recorded in the FT mode, and T_1 was obtained by the $\pi-\tau-\pi/2$ pulse sequence. The ^{14}N chemical shifts of DPPC and EPC vesicles and of 3 M $N(CH_3)_4Br$ aqueous solution were measured from the signal position of a 5.4 M NH_4Cl aqueous solution. The chemical shifts of EPC and of CTAB were measured in methanol in nonaggregated states. ^{1}H NMR spectra were obtained at 100 MHz with a JEOL-PS-100 spectrometer and a JNM-VT-3T.

Results

^{1}H NMR of DPPC vesicles (not shown) gave two $-N^+(CH_3)_3$ signals, 0.02 ppm apart from each other, corresponding to the inside and the outside positions in a vesicle. The broadening out of chain methylene signals below the chain melting point T_m was obvious. The formation of single bilayer vesicles was confirmed by reference to the work of Sheetz & Chan (1972).

The ^{14}N signal of $-N^+(CH_3)_3$ in DPPC or in EPC vesicles could be simulated by a single Lorentzian (Figure 1) showing itself in the motional narrowing region. A small shift difference between inside and outside signals, if present, must be masked within the line width. The signal inversion recovery process could always be simulated by a single exponential function of time τ as shown in Figure 1B.

^{14}N relaxation times, T_1 and T_2, are plotted against $1/T$ in Figure 2, the half full width $\Delta\nu_{1/2}$ of the signal being taker

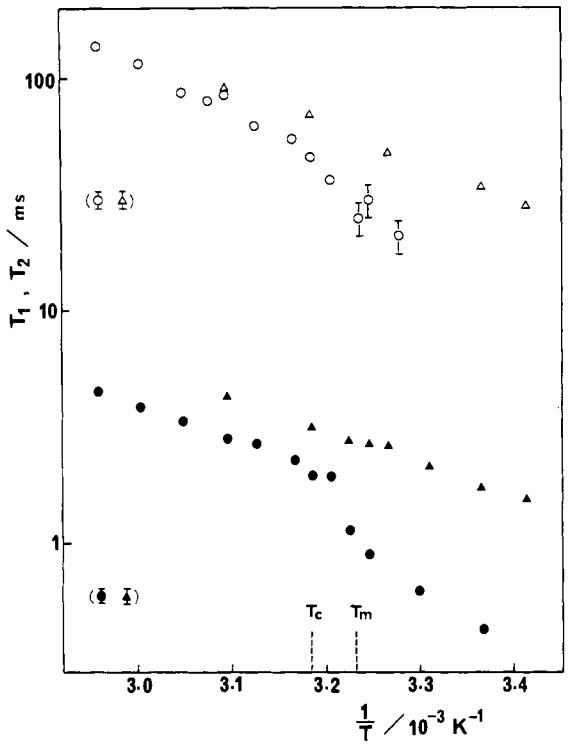

FIGURE 2: ^{14}N relaxation times T_1 and T_2 vs. $1/T$ for lecithins in single bilayer vesicles: (○) T_1 for DPPC; (●) T_2 for DPPC; (△) T_1 for EPC; (▲) T_2 for EPC. T_c and T_m are the chain melting points of DPPC for multilamellar dispersions and single bilayer vesicles, respectively (Lentz et al., 1976).

as the measure of T_2 ($T_2^{-1} = \pi\Delta\nu_{1/2}$). T_2s were always shorter than T_1s by factors of 15–35 in vesicles. This was in contrast to the observation that T_2s were only slightly shorter than T_1s in the case of inverse micelles of EPC in various organic solvents with a small amount of water (K. Koga and Y. Kanazawa, unpublished experiments) where the micelle radius was small (Elworthy & McIntosh, 1964). There was a jump in the T_2 slope of DPPC vesicles near T_m, while such a discontinuity was less clear in T_1 of the same sample.

The ^{14}N chemical shift of DPPC and that of EPC in vesicles coincided, and they were 4 ppm downfield from 3 M $N(CH_3)_4Br$ aqueous solution without temperature dependence under the experimental conditions.

In order to evaluate the effect of the glycerophosphoryl group attached close to the trimethylammonium ion on the electronic environment of the nitrogen nucleus, the ^{14}N chemical shifts of trimethylammonium ions in EPC and in CTAB were measured in methanol at 29 °C. By their narrow widths, 5 Hz for EPC and 3 Hz for CTAB, it was indicated that neither of these molecules was seriously aggregated in this solvent, giving precise measurements of the shifts. The signal height of a mixed solution was additive within an experimental error of 5%; hence, there was no marked shift difference between these compounds.

Data Analysis

Two kinds of molecular motions reorienting the principal axis of the electric field gradient tensor at the nitrogen nucleus, the C_β–N director, are assumed to be effective for the ^{14}N quadrupole relaxation. One is the motion along the surface

$$-O\overset{\alpha}{C}H_2-\overset{\beta}{C}H_2-\overset{\gamma}{N}(CH_3)_3$$

of the aggregate due to the tumbling motion of the whole aggregate and to the lateral diffusion of the molecules around the vesicle characterized by a correlation time τ_R. The other is the local molecular or segmental motion with a short correlation time, τ_r, very likely to be under the extreme narrowing condition of NMR relaxation, $\omega_0\tau_r \ll 1$, where ω_0 is the Larmor frequency, 4.5×10^7 s^{-1}. Familiar relations for the nuclear quadrupole relaxation (Abragam, 1961) with small modifications are used accordingly:

$$1/T_1 = (8\pi^2/15)\nu_Q^2\{[\tau_R/(1 + \omega_0^2\tau_R^2) + 4\tau_R/(1 + 4\omega_0^2\tau_R^2)]S^2 + 5\tau_r f(S)\} \quad (1)$$

$$1/T_2 = (8\pi^2/15)\nu_Q^2[(3\tau_R/2 + 5\tau_R/\{2(1 + \omega_0^2\tau_R^2)\} + \tau_R/(1 + 4\omega_0^2\tau_R^2))S^2 + 5\tau_r f(S)] \quad (2)$$

where $\nu_Q = (3/4)\{I(2I - 1)\}^{-1}(e^2qQ/h) = 3e^2qQ/4h$ is the strength of the quadrupole interaction for $I = 1$ nucleus, S is the order parameter of the C_β–N director with respect to the bilayer normal, and $f(S)$ is the factor correcting the effect of anisotropic behavior of the local motion and may be regarded as unity when $|S|$ is very small compared with 1. Equations 1 and 2 can be rewritten as

$$1/T_1 = (8\pi^2/3)\nu_Q^2[\tau_r f(S) + S^2F(\tau_R)/5] \quad (3)$$

$$1/T_2 - 1/T_1 = (4\pi^2/5)\nu_Q^2S^2G(\tau_R) \quad (4)$$

with

$$F(\tau_R) = \tau_R[1/(1 + \omega_0^2\tau_R^2) + 4/(1 + 4\omega_0^2\tau_R^2)]$$

$$G(\tau_R) = \tau_R[1 + 1/(1 + \omega_0^2\tau_R^2) - 2/(1 + 4\omega_0^2\tau_R^2)]$$

and $G(\tau_R)$ can be replaced by τ_R within an error of 0.5% if τ_R is longer than 2×10^{-7} s.

For the present data analysis, the values of order parameter S (= $S_{C_\beta-N}$) are obtained from the $-N^+(CD_3)_3$ deuteron NMR splitting of the DPPC lamellar system (Galley et al., 1975) and those of EPC multilamellar dispersions with the additional information that the change in the system from multilamellar dispersion to single bilayer vesicles has not affected the order parameter (Stockton et al., 1976). The relation between order parameters, $|S_{C_\beta-N}| = 10.8|S_{C_\gamma-D}|$, used for $|S_{C_\beta-N}|$ evaluation (Gally et al., 1975) must be correct, since neither the symmetry axis $-N^+(CD_3)_3$ nor $-CD_3$ is expected to be distorted. Since the resultant $|S_{C_\beta-N}|$ is smaller than 0.16, $f(S)$ may be replaced by 1. It has been shown that the phase transition (gel–liquid crystal) range is wide in single bilayer vesicles, and the range observed for DPPC with the DPH probe method is 29.7–40.6 °C with $T_m = 36.4 \pm 0.5$ °C (Lentz et al., 1976). The gel-phase order parameter S_g can confidently be used for vesicles at 24 °C and approximately at 30 °C. The liquid-crystal phase order parameter S_l can be used above 41 °C. Intermediate values should be taken within the transition region.

The value $\nu_Q = 83.5$ kHz obtained for the ^{14}N nucleus of $-N^+(CH_3)_3$ in *n*-hexyltrimethylammonium bromide (HTAB) has been used for the analysis of CTAB (Henriksson et al., 1977) under the assumption that the difference in the alkyl chain lengths does not have much influence on the electric field gradient at the nitrogen nucleus when the chains are long enough. Since no shift difference was observed between EPC and CTAB in the present experiment, the electronic environment of the nitrogen nucleus in phosphatidylcholine and that in CTAB must be alike. This result gives a good basis for the approximation of using ν_Q of HTAB for phosphatidylcholine (see Mason, 1976). It is also assumed that the electronic environment is not seriously perturbed by the process of aggregation.

The correlation times for the local motions and their activation energies are listed in Table I.

The correlation time τ_R can be determined by the relation

Table I: Correlation Times (τ_r) and Activation Energies (E) for the Molecular Motions by Nuclear Relaxation Data

lipid	state[a]	position	temp (°C)	τ_r (ns)	E (kJ·mol^{-1})	nucleus
DPPC	V	C_β-N	32-65	$0.24\text{-}0.03_9$	36[b] ± 4	^{14}N[e]
	M	C_α-D	46-76		21	^2H[f]
	M	C_γ-D	44-67	0.033-0.019	16	^2H[f]
EPC	V	C_β-N	20-50	$0.19\text{-}0.06_0$	31[c] ± 1	^{14}N[e]
	V	C_γ	50	0.020		^{13}C[g]
	V	C_γ-D	30	0.05[d]		^2H[h]
	D	C_γ-D	30	0.05[d]		^2H[h]

[a] V, single bilayer vesicle; M, multilamella; and D, multilamellar dispersion. [b] Obtained for the data above 43 °C. [c] Obtained for the whole temperature region. [d] Calculated with T_1 data in the reference by the relation $1/T_1 = 3\pi^2/2(e^2qQ/h)^2\tau_r$ and e^2qQ/h = 170 kHz. [e] This work. [f] Gally et al. (1975). [g] Gent & Prestegard (1977). [h] Stockton et al. (1976).

$1/\tau_R = 1/\tau_a + 1/\tau_d$ (Bloom et al., 1975), where $\tau_a = 4\pi a^3\eta/3kT$ is the correlation time for the vesicle tumbling motion evaluated according to the Stokes–Einstein model, with the radius of particle a and the viscosity of the medium η. $1/\tau_d = 6D/R^2$ is the term due to the translational diffusion of the molecule (diffusion coefficient D) along the vesicle surface of radius R.

The Stokes radius of EPC vesicles prepared by the ultrasonic method has been determined to be 105.6 Å at 20 °C by diffusion measurement and the Stokes–Einstein–Sutherland equation (Newman & Huang, 1975). A similar value of 106 Å has been reported also for EPC at 20 °C (Roseman et al., 1978). The correlation time for the vesicle tumbling motion τ_a at 20 °C can then be calculated to be 1.22 μs. The combination of values ν_Q = 83.5 kHz, $|S|$ = 0.106,[2] and $1/R^2 = 2/3R_{out}^2 + 1/3R_{in}^2$, a weighted average value,[3] results in the lateral diffusion coefficient $D = 2 \times 10^{-8}$ cm^2 s^{-1}. This is in excellent agreement with the observed results in EPC by a variety of methods: 1.8×10^{-8} cm^2 s^{-1} at 25 °C in oriented lamella by spin-label ESR (Devaux & McConnell, 1972), 3.0×10^{-8} cm^2 s^{-1} at 20 °C in vesicles by fluorescence probe (Vanderkooi & Callis, 1974), and 4×10^{-8} cm^2 s^{-1} at 31 °C in vesicles by ^{1}H NMR (Bloom et al., 1978).

The diffusion coefficient of DPPC below T_m has been found to be smaller than 2×10^{-9} cm^2 s^{-1} irrespective of the method of observation (^{1}H NMR by Lee et al. (1973), photobleaching recovery by Wu et al. (1977) and Rubenstein et al. (1979), and fluorescence probe by Fahey & Webb (1978)). Under such conditions, the contribution of the diffusion term to τ_R is negligible, and it is possible to evaluate the vesicle radius under the approximation $\tau_R \simeq \tau_a = 4\pi a^3\eta/3kT$. The calculation with our τ_R gives a = 120 Å at 24 °C and 115 Å at 30 °C, indicating that the radius decreases as the temperature is raised toward the phase transition region. It is noteworthy that the above result agrees with that found for DMPC vesicles (Watts et al., 1978), where the Stokes radius has been found to decrease toward T_m when the temperature is raised (it increases again above T_m). The diffusion coefficient of DPPC at 50 °C is calculated for the estimated Stokes radius of 125 Å (117 Å), the maximum (minimum) estimation of the hydration layer of 13 Å (5 Å)[4] added to the vesicle radius of 112 Å obtained by ^{31}P NMR (de Kruijff et al., 1976). The resultant D is 4 (2) $\times 10^{-8}$ cm^2 s^{-1}, which is again reasonable in magnitude.[5] In the phase transition region (29.7–40.6 °C) it is expected that the diffusion process will take place to some extent, and the order parameter will take a value in between S_g and S_l', where S_l' is the extrapolated value from the liquid crystal region. Trial calculation gave the following results. (1) When small diffusion coefficients were employed throughout the transition region according to ^{1}H NMR (Lee et al., 1973; Fisher & James, 1978), where D has not exceeded 4×10^{-9} cm^2 s^{-1} even at 41 °C, $|S|$ and a were directly connected: the vesicle radius was in between 110 Å (S_g = 0.144) and 126 Å (S_l' = 0.117) at 35 °C, in between 103 Å (S_g = 0.141) and 120 Å (S_l' = 0.113) at 37 °C, and approximately 104 Å (S_l' = 0.110) at 39 °C. The vesicle radius was to decrease toward the completion of phase transition. This result did not agree with that of DMPC (see above). (2) When the liquid-crystal value of the order parameter S_l' was taken and the radius was supposed to increase above T_m as in the case of DMPC, a large value of D should be expected even before the completion of phase transition at 39 °C; for example, $D = 2 \times 10^{-8}$ cm^2 s^{-1} (a = 110 Å) and $D = 4 \times 10^{-8}$ cm^2 s^{-1} (a = 115 Å).

Discussion

It is important to develop a suitable technique to measure the dynamical properties of lipid molecules in membranes such as a lateral diffusion coefficient and the parameters for local molecular motions for the study of biological phenomena where the changes in the physical properties of membranes are significant. The technique is ideal if it requires no system modification, especially for studies under the presence of the sources of system perturbations. Each method that has been used for the evaluation of D has involved assumptions and/or approximations inherent in the technique, and the values of D have been spread in a wide range. For example, the modification of the system is not required for an NMR study, but some assumptions are unavoidable for the data analysis, especially when the dipolar relaxation is dominant. On the other hand, a large value in D seems to be given by the method in which modified lipids or nonlipid probes were used. Wu et al. (1977) have reported that the diffusion coefficients by fluorescent probe molecules diO-C_{18} and NBD-PE, measured in an identical host (oriented lamella of EPC), have differed by a factor of 4. The effect of probe molecules on the observed D is obvious, but it is difficult to judge whether the effect is due to the perturbation of the whole system by the insertion of the probe or to the different behaviors of observed probes from the host lipids.

Throughout the present analysis of ^{14}N relaxation times, the use of ν_Q of HTAB (83.5 kHz) provides reasonable results for the magnitude in both τ_r and D.

The activation energies for the local motion of the C_β–N bond obtained from τ_rs are 36 kJ·mol^{-1} for DPPC above T_m

[2] The numerical representation of S is given to three significant figures simply in order to show its variation where necessary, and its absolute value might not be reliable that far.

[3] The weight is determined according to the ratio of numbers of lipid molecules on the outer and inner monolayers of the vesicles: n_{out}/n_{in} = 2.0 (de Kruijff et al., 1976) and 2.1 (Huang & Mason, 1978).

[4] The thicknesses of outer hydration layers are 13.3 and 4.8 Å for the DMPC vesicle at 30 and 25 °C, respectively (Watts et al., 1978), and 6 Å for EPC at 20 °C (Huang & Mason, 1978).

[5] Ds in DPPC above T_m reported recently are as follows: $3.1\text{–}6.1 \times 10^{-9}$ cm^2 s^{-1} (47–67 °C) in vesicles by ^{1}H relaxation (Lee et al., 1973), $3.8\text{–}5.3 \times 10^{-9}$ cm^2 s^{-1} (45–74.5 °C) in multilamella by ^{1}H $T_{1\rho}$ (Fisher & James, 1978), 1.0×10^{-8} cm^2 s^{-1} in vesicles and 0.4×10^{-8} cm^2 s^{-1} in oriented lamella by the fluorescence photobleaching method with diI (Fahey & Webb, 1978), 10.1×10^{-8} cm^2 s^{-1} (48 °C) in oriented lamella by the photochemical technique with head-group spin-labeled phospholipid (Sheats & McConnell, 1978), and $<6\text{–}19 \times 10^{-8}$ cm^2 s^{-1} (43.5–61 °C) in oriented lamella with 40% (w/w) D_2O/DPPC by ^{1}H spin–echo (Kuo & Wade, 1979).

and 31 kJ·mol^{-1} for EPC vesicles, which are obviously larger than 21 kJ·mol^{-1} for the C_α–D motion in $POCD_2CH_2N^+$ of the DPPC lamellar phase (Gally et al., 1975). Since there is no reason to expect that the head group in a vesicle is more restricted in motion than in the lamellar phase, the origin of the observed larger activation energy for C_β–N motion than that for C_α–D should be found in the environments of the bonds. The $N^+(CH_3)_3$ group, although located at the end of the molecule, must be susceptible to an appreciable motional restriction in the network of negative PO_4^- and positive $N^+(CH_3)_3$ groups. This is consistent with the data of order parameter $|S_{C_\beta\text{-}N}| \simeq 3|S_{C_\beta\text{-}D}|$ (Gally et al., 1975) indicating that the rotation around the C_α–C_β bond is restricted. It is also consistent with the result that phosphorus and nitrogen atoms lie in the same plane (Seelig et al., 1977). The larger activation energies of τ_rs observed for the DPPC vesicle in the phase transition region must be due to the effect of the change in the dynamical properties in the chain. The quantities τ_r and E of the local motion will serve as good measures of the intermolecular interactions between vesicle constituents and other substances, especially of those with an electrostatic effect.

Since the diffusion coefficient is evaluated in the form of subtraction, $1/\tau_d = 1/\tau_R - 1/\tau_a$, and the term τ_a is usually dominant, we should admit that the absolute value of D involves a fair amount of error. Nevertheless, the diffusion coefficient 2×10^{-8} cm^2 s^{-1} evaluated for EPC at 20 °C with $a = 105.6$ Å has a magnitude consistent with the Ds obtained by various methods. This is also true for the diffusion coefficient of DPPC.

It is of interest that the vesicle size of DPPC decreases with increasing temperature below the start of phase transition as in the case of DMPC. This can be the result of the change in the molecular packing accompanied by a phenomenon similar to that of pretransition known for multilamellar systems.

Although the present analysis has been done with insufficient Stokes radii data, allowing a fair amount of uncertainty in the lateral diffusion coefficient, it is shown that this method provides reliable information on local motions and can be used for sensitive detection of the change in the diffusion process in small vesicles including the phase transition region without system modification. The method of ^{14}N relaxation rates can be a very important technique for the study of membrane perturbations at the molecular level.

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