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ANOMALIES OF NANOSECOND ULTRASONIC RELAXATION IN THE LIPID BILAYER TRANSITION

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The frequency dependence of ultrasonic velocity as well as absorption in a suspension of sonicated dipalmitoylphosphatidylcholine vesicles was measured by a differential ultrasonic resonator. The frequency was scanned between 1.3 and 13 MHz and the temperature was varied from 25 to 47°C. A pronounced relaxation was observed in the time range of 10 ns. The data were analyzed assuming a single relaxation which appeared to be a good approximation. The relaxation time as well as relaxation strength increased anomalously in the vicinity of the gel-to-liquid crystal transition of 41.5°C. This result represents the first definite evidence of the critical slowing down in the lipid bilayer and is discussed in terms of the Landau theory of phase transition. The possible biological significance of the mechanical relaxation is also presented.

Introduction

It has been established that biological membranes are not rigid bodies but flexible and fluid materials. After Frye and Edidin [1] elucidated the fluid nature of biological membranes, molecular dynamics of membranes were extensively studied by various techniques: fluorescence measurements [2,3], absorption anisotropy decay [4], electron spin resonance [5,6] and so on. The mobility of probe molecules in membranes as measured by these techniques was rather high. For example, the rotational relaxation time of lipid-like molecules is about 1 ns [3], and their translational diffusion constant is about 10^{-8} cm^2/s [5,6]. So, lipid bilayers in biological membranes are two-dimensional liquids, in which molecular diffusion may freely occur in the plane of membrane and not in a perpendicular direction.

It should be noted that these techniques of molecular dynamics are concerned with the dynamic properties of a single molecule which are influenced by local environment. Therefore, the dynamics of membranes measured by these techniques have to represent a short-range molecular fluctuation which is rather insensitive to long-range structural fluctuation. However, thermodynamic fluctuation of membrane order in semi-microscopic domains is also expected in addition to the molecular movement. Thermodynamic fluctuation of membrane order is particularly interesting in the vicinity of the transition temperature, because an anomalous enhancement of the relaxation time and strength is expected from the universality of transition behaviors. In general, second-order phase transition is characterized by the disappearance of the restoring force against the fluctuation of order parameter, irrespective of the physical nature of the system [7,8]. As a result of this fact, the magnitude, the relaxation time and the characteristic length of structural fluctuation markedly increases in the neighborhood of the transition temperature. It was previously suggested from the ultrasonic velocity at 3 MHz that the

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phase transition of pure phosphatidylcholine membrane is very near a second-order transition [9,10]. Theoretical calculation also predicted a second-order character of the lipid bilayer transition [11–13]. If we can measure the relaxation time as well as strength of thermodynamic fluctuation of membrane order, it is possible to prove decisively the second-order character of the lipid bilayer transition.

In the present work, we have measured the frequency dispersion of ultrasonic velocity and absorption of a lipid bilayer suspension by a differential ultrasonic resonator [14,15], because the ultrasonic propagation is very sensitive to thermodynamic structural fluctuation in the vicinity of phase transition point [16,17]. It was elucidated that there was a relaxation of about 10 ns in lipid bilayers, which anomalously increased in the vicinity of the gel-to-liquid crystal transition. This fact together with anomalous enhancement of the relaxation strength clearly indicated the second-order character of the lipid bilayer transition.

Materials and Methods

Synthetic L-α-dipalmitoylphosphatidylcholine was purchased from Sigma Chemical Co. and used without further purification. First, multilamellar liposomes of dipalmitoylphosphatidylcholine were prepared by stirring the lipid film with distilled water in a round-bottomed flask. Then, single lamellar small vesicles were obtained by sonicating the multilamellar liposomes for 30 min at the power level of 150 W [9]. We used the suspension of single lamellar vesicles with the dry weight concentration of 5.0 mg/ml.

We have developed a differential ultrasonic resonator which is specially designed for the frequency dispersion measurements in dilute membrane suspensions [14,15]. The principle of the apparatus is the following. A steady ultrasonic wave is generated in a cell by the sending transducer. The output voltage from the receiving transducer is monitored by a high-frequency voltmeter, which varies according to the frequency due to the resonance conditions in the cell. When the ratio of the cell length to the ultrasonic wavelength is equal to a half integer, reflected ultrasonic waves interfere with each other to give a maximum value of output voltage. Hence, the ultrasonic velocity is determined from the frequency of resonance peaks, whereas the ultrasonic absorption is obtained from the half-width of the resonance peak.

Since high accuracy is necessary for the measurements of dilute membrane suspensions, we used a differential type of cells and developed a new method of analysis. The output voltage U is measured as a function of the frequency f of the input signal. The theoretical relationship between U and f is described by

$$U = U_{\max}\left(1 + \frac{\sin^2(2\pi fl/V)}{\sinh^2(\alpha l)}\right)^{-1/2} \tag{1}$$

where $U_{\max}$ is the output voltage at the maximum, l is the cell length, V and α are the ultrasonic velocity and absorption [15,18], respectively. If we fit Eqn. 1 to experimental data by a least-square method assuming that V and α are unknown parameters, very accurate determination of the ultrasonic velocity as well as absorption is possible. In fact, we could determine the ultrasonic velocity and absorption per wavelength to an accuracy of ± 1 cm/s and $\pm 2 \cdot 10^{-4}$ when 20 points in the vicinity of a resonance peak were used for the least-square fitting. Differential cells were employed in order to cancel the large temperature coefficient of the ultrasonic velocity in water, which was particularly necessary for dilute suspensions. The frequency range was between 1 and 13 MHz when the absorption was small enough. When the ultrasonic absorption became large, the frequency range was reduced to between 1.5 and 8 MHz. Although the frequency range of our measurements is rather narrow, the accurate measurements of both ultrasonic velocity as well as absorption enabled us to determine the relaxation time and strength unambiguously. Details of the apparatus are presented elsewhere [15].

When the medium of ultrasonic propagation shows a single relaxation, the ultrasonic velocity as well as absorption per wavelength are expressed by the following equations,

$$V^2 = V_0^2 + \left(V_\infty^2 - V_0^2\right)\frac{\omega^2\tau^2}{1+\omega^2\tau^2} \tag{2}$$

$$\alpha\lambda = \frac{\pi\left(V_\infty^2 - V_0^2\right)}{V^2}\left(\frac{\omega\tau}{1+\omega^2\tau^2}\right) \tag{3}$$

in which V_∞ and V_0 are the ultrasonic velocity at high- and low-frequency limits, and τ is the relaxation time [19]. If we measure the ultrasonic velocity as well as absorption of homogeneous medium as a function of frequency, we can determine the relaxation time and strength by Eqns. 2 and 3.

In the case of membrane suspensions, however, the ultrasonic velocity and absorption depends on the concentration of membranes. Furthermore, the ultrasonic velocity of a suspension is severely affected by the change in the ultrasonic velocity of aqueous solvent. Therefore, we use the limiting numbers of velocity $[V]$ and absorption per wavelength $[\mu]$ [20]:

$$[V] = \lim_{c \to 0} \frac{V - V_s}{V_s c} \tag{4}$$

$$[\mu] = \lim_{c \to 0} \frac{\mu - \mu_s}{c} = \lim_{c \to 0} \frac{\alpha\lambda - \alpha_s\lambda_s}{c} \tag{5}$$

On the assumption that the suspension is very dilute, namely the difference between suspension and solvent is small, we can derive simple equations of ultrasonic relaxation of suspensions:

$$[V] = [V]_0 + \Delta \frac{\omega^2\tau^2}{1 + \omega^2\tau^2} \tag{6}$$

$$\frac{[\mu]}{2\pi} = \Delta \frac{\omega\tau}{1 + \omega^2\tau^2} \tag{7}$$

The relaxation strength Δ is described by

$$\Delta = \tfrac{1}{2} \lim_{c \to 0} \frac{V_\infty^2 - V_0^2}{V_s^2 c} = [V]_\infty - [V]_0 \tag{8}$$

where $[V]_\infty$ and $[V]_0$ in this case denote the limiting velocity number of a membrane suspension at high- and low-frequency limits and c is the dry weight concentration of membranes. Since the relaxation strength Δ is an intrinsic quantity of suspended membranes, comparison of various data becomes possible by Eqns. 6, 7 and 8.

Results

We have measured the frequency dependence of the ultrasonic velocity in the temperature range from 25 to 47°C. Figs. 1, 2 and 3 represent the results at 30.8°C, 39.9°C and 41.4°C, respectively, as examples. Corresponding data of ultrasonic absorption were reported by Gamble and Schimmel [21] for these three points. They reported parameters of best fitted single relaxation curves. The limiting number of absorption per wavelength was calculated from their parameters and is shown by dashed lines in the frequency range above 15 MHz where their measurements were carried out. Solid lines in Figs. 1–3 represent single relaxation curves fitted by Eqns. 6 and 7. The estimated relaxation time and strength are shown in Table I. Our data and the data of Gamble and Schimmel agreed fairly well with single relaxation curves. The relaxation time and strength which Gamble and Schimmel obtained from their data were smaller than ours. This is mainly due to the fact that they

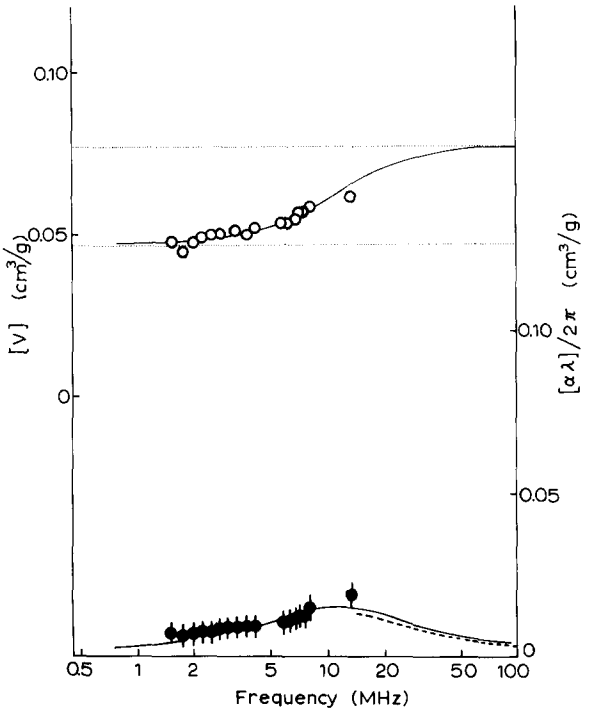

Fig. 1. The limiting numbers of velocity (○) and absorption per wavelength (●) at 30.81°C of a suspension of sonicated dipalmitoylphosphatidylcholine vesicles are plotted as a function of frequency. A differential ultrasonic resonator was used for the measurement. The dry weight concentration of the sample was 5 mg/ml. Distilled water was used as solvent. The dashed line represents the data of Gamble and Schimmel [21] at 30°C. Solid lines are the best fitted single relaxation curves.

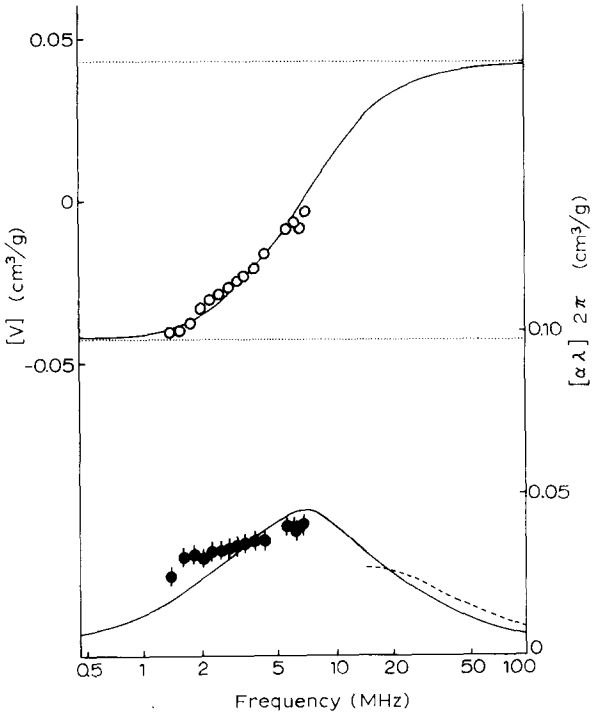

Fig. 2. The limiting numbers of velocity (○) and absorption per wavelength (●) at 39.90°C of a suspension of sonicated dipalmitoylphosphatidylcholine vesicles are plotted as a function of frequency. The conditions of measurement are the same as Fig. 1. The dashed line represents the data of Gamble and Schimmel [21] at 40°C. Solid lines are the best fitted single relaxation curves.

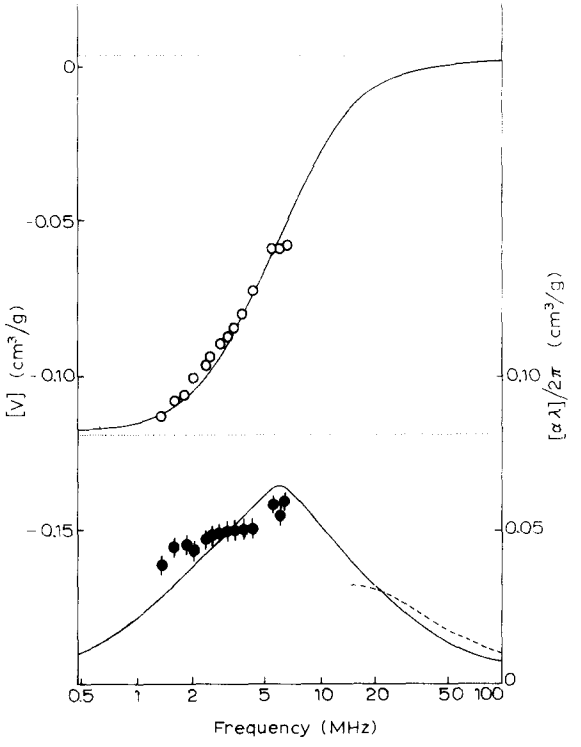

Fig. 3. The limiting numbers of velocity (○) and absorption per wavelength (●) at 41.45°C of a suspension of sonicated dipalmitoylphosphatidylcholine vesicles are plotted as a function of frequency. The conditions of measurement are the same as Fig. 1. The dashed line represents the data of Gamble and Schimmel [21] at 41.3°C. Solid lines are the best fitted single relaxation curves.

measured only ultrasonic absorption in the frequency range above the relaxation frequency. We measured both ultrasonic velocity and absorption. Furthermore, we compared our data with the data of Gamble and Schimmel. Therefore, there is little ambiguity in the relaxation parameters of our analysis.

It is apparent from Figs. 1–3 that there is a relaxation at about 10 MHz. The relaxation time as well as strength are enhanced when the temperature increases to the transition point in the gel phase. The same tendency was also observed in the liquid-crystalline phase above the transition temperature. That is, the relaxation time and strength increased abruptly, when the temperature is decreased and approaches to the transition point.

The single relaxation curve is in good agreement with the experimental data, when the tem-

TABLE I

RELAXATION PARAMETERS OF DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES

Temp. (°C)	τ (ns)	Δ (cm^3/g)	$\delta\kappa_m$ (cm^2/dyn) ($\times 10^{11}$)
25.02	14±2	0.0250	0.22
30.81	15	0.0300	0.26
34.15	17	0.0421	0.37
37.98	22	0.0680	0.59
39.90	24	0.0860	0.75
41.08	26	0.1226	1.05
41.49	29	0.1224	1.05
42.29	23	0.0894	0.77
43.99	21	0.0335	0.22
46.59	15	0.0164	0.14

perature is far from the transition point. However, it appears that there is distribution of relaxation time in the vicinity of the transition temperature. The accuracy and the frequency range of our measurements is not so good at present as to be analyzed by multiple relaxation. So, we neglect here the possible distribution of the relaxation time. This simplification does not affect the semi-quantitative discussions about the relaxation time and strength.

Fig. 4 shows the temperature dispersion of the ultrasonic velocity at 2, 4, 6 and 8 MHz. Dashed lines represent $[V]_\infty$ and $[V]_0$, as analyzed by a single relaxation formula. At the low-frequency limit, $[V]_0$ shows a very sharp dip near the transition point, whereas $[V]_\infty$ monotonously decreases and the inclination becomes sharper only in the vicinity of the transition temperature.

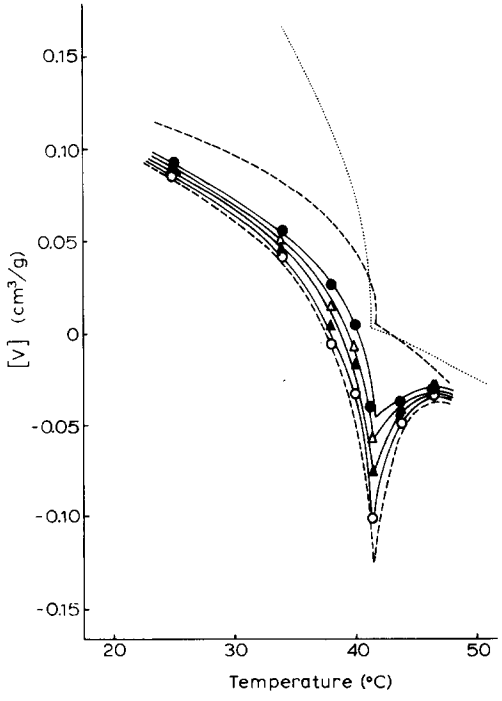

Fig. 4. The limiting velocity number at 2 MHz (○), 4 MHz (▲), 6 MHz (△) and 8 MHz (●) are plotted as a function of temperature. Dashed lines represent the limiting velocity numbers at the high-frequency limit $[V]_\infty$ (upper) as well as at low-frequency limit $[V]_0$ (lower), respectively. A dotted line represents the limiting velocity number in the frequency range of GHz, which is calculated from the data of Brillouin light scattering [37].

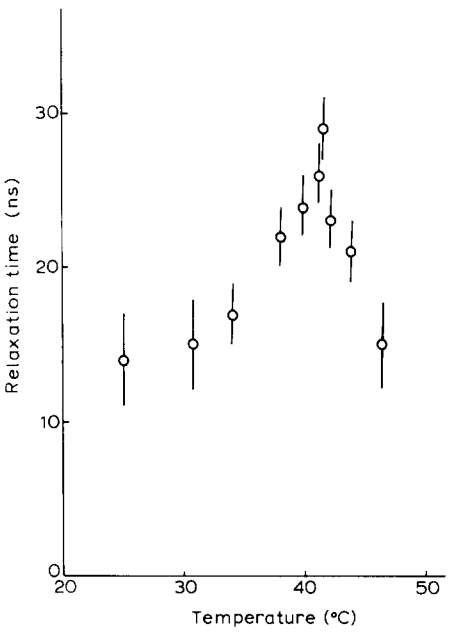

Fig. 5. Relaxation time plotted as a function of temperature.

The temperature dependence of the relaxation time as well as relaxation strength are shown in Figs. 5 and 6. Remarkable enhancement of these

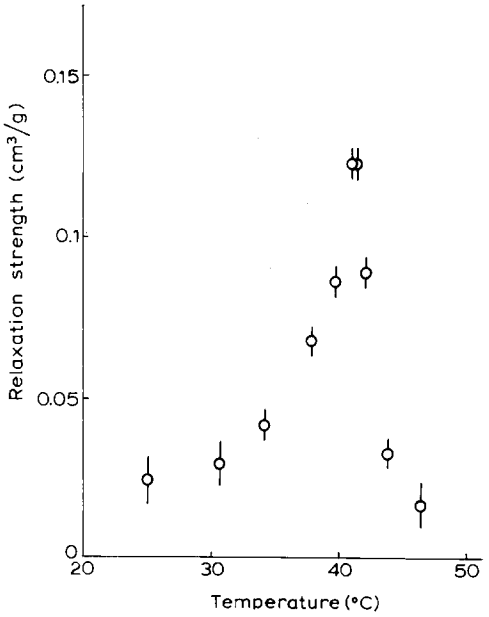

Fig. 6. Relaxation strength plotted as a function of temperature.

quantities is obtained in the vicinity of the transition temperature. The relaxation time below 30°C and above 46°C is about 15 ns. It increases anomalously to about 30 ns at the transition temperature. The relaxation strength also increases from about 0.02 to 0.12 cm^3/g at the transition temperature. The width of the transition anomalies is very broad and more than ten degrees.

Discussion

The experimental results are summarized as follows: (1) The ultrasonic relaxation of lipid bilayers is characterized by a relaxation time of the order of 10 ns. (2) The relaxation time as well as strength of this relaxation anomalously increase in the vicinity of the transition point. (3) The limiting velocity number at low-frequency limit has a distinct dip, whereas the limiting velocity number at high-frequency limit only shows a break point at the transition temperature.

Several questions arises from these results: Which kind of molecular process is associated with the ultrasonic relaxation? Why and how are the relaxation time and relaxation strength enhanced in the vicinity of the transition temperature? The temperature dispersion of the velocity appears to be qualitatively different from the sigmoidal temperature dependence of mechanical properties estimated by the techniques of molecular dynamics [22,23]. How is this apparent disagreement explained? Do the transition anomalies have any biological significance?

The first two questions are concerned with the molecular mechanism of the ultrasonic relaxation in the lipid bilayer. We discuss this problem on the basis of the Landau theory of phase transition. In the framework of the Landau theory, it is assumed that the free energy of the system may be expanded by an order parameter in the vicinity of the transition temperature:

$$F(P,T,\eta) = F_0(P,T) + \tfrac{1}{2}A_2\eta^2 - \tfrac{1}{3}A_3\eta^3 + \tfrac{1}{4}A_4\eta^4 \tag{9}$$

$$A_2(P,T) = a(P)(T - T^*) \tag{10}$$

Here, η is the order parameter and T^* is the lower pseudo-critical temperature. The coefficients a, A_3, A_4 are positive and only weakly dependent on the temperature. This type of free energy was successfully used for the analysis of the nematic-isotropic transition, in which η represents the orientational order parameter [25,26]. The validity of Eqn. 9, however, is not self-evident for the lipid bilayers, because we do not know what kind of parameter best describes the order of membranes. The plausible alternatives of the membrane order are the average orientational order of hydrocarbon chains [27,28] and the lateral packing density [29]. As a matter of fact, the average orientational order abruptly decreases at the transition temperature [30], which is analogous to the nematic-isotropic transition. On the other hand, the lateral packing density is considered to be a relevant parameter not only because it shows a sharp decrease [31] but also because the lateral pressure-area curves are very similar to the pressure-volume curves in a gas-liquid transition [32–34]. Due to the ambiguity of the order parameter mentioned above, we here assume only that the free energy of the lipid bilayer may be expanded by a certain order parameter, η, as per Eqns. 9 and 10. This assumption has been used successfully for many physical systems.

Then, we can derive various features of the transition. The transition temperature T_c is defined by

$$F(P, T_c, \eta_c) = F_0(P, T_c) \tag{11}$$

When the temperature is lower than the transition temperature T_c, the order parameter is finite, whereas $\eta = 0$ in the disordered phase. The temperature T^* corresponds to the point at which the disordered phase becomes absolutely unstable. When $A_3 = 0$, T^* coincides with the transition temperature T_c and the transition becomes of second order. It may be deduced as well from the consideration of order parameter fluctuation that the magnitude $\langle \delta\eta^2 \rangle$, the relaxation time τ and the coherence length ξ of the fluctuation of the order parameter are enhanced near the transition temperature [7,25,28],

$$\langle \delta\eta^2 \rangle = \frac{kT}{a(T - T^*)} \tag{12}$$

$$\tau = \frac{\zeta}{a(T - T^*)} \tag{13}$$

$$\xi = \xi_0 \left(\frac{T^*}{T - T^*} \right)^{1/2} \tag{14}$$

Here, ζ is a phenomenological kinetic coefficient. Eqns. 12–14 indicate that $\langle \delta\eta^2 \rangle$, τ and ξ increase anomalously in the vicinity of the transition temperature (exactly speaking, the pseudo-critical temperature T^*).

The remarkable slowing down of the ultrasonic relaxation time in Fig. 5 seems to indicate the applicability of Eqn. 13 at least qualitatively to the gel-to-liquid crystal transition. However, in order to conclude that the present results certainly arise from the slowing down of the order parameter fluctuation, we have to prove following propositions. (1) The ultrasonic propagation is certainly coupled with the order parameter. (2) The magnitude of the relaxation time is reasonable for the order parameter fluctuation.

Since a longitudinal wave causes adiabatic compression, its propagation is subject to coupling with thermal properties of the medium. The anomalous behavior of the ultrasonic propagation was first discussed by Landau and Khalatnikov [24] and developed by Okano and coworkers [26] on the assumption of Eqns. 9 and 10. The basic idea of their theory is as follows. Ultrasonic propagation gives rise to periodic changes of local pressure and temperature. Thereby, the equilibrium values of the free energy, the order parameter and so on vary periodically. Due to the friction to the change in the order parameter, we will have a phase difference between the periodic changes of pressure (or temperature) and the order parameter over a certain frequency range. That is, the ultrasonic velocity and absorption show relaxation. If we assume that A_3 is zero, the adiabatic compressibility κ^s is easily calculated [26] as:

$$\kappa^s(i\omega) = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right) = \kappa_0^s + \delta\kappa_m \left(\frac{1}{1 + i\omega\tau} \right) \tag{15}$$

$$\kappa_0^s = \kappa_0^T - \left(TV\theta_0^2 / C_{p,0} \right) \tag{16}$$

$$\tau = \frac{\zeta}{a\left(\left(Ta^2 / C_{p,0} A_4 \right) + 2 \right)} \left(\frac{1}{T_c - T} \right) \tag{17}$$

$$\delta\kappa_m = \frac{\left(\left(TV\theta_0 a / C_{p,0} \right) + b \right)^2}{V\left(\left(Ta^2 / C_{p,0} A_4 \right) + 2 \right) A_4} \tag{18}$$

Here, κ_0^T, θ_0 and $C_{p,0}$ are the isothermal compressibility, thermal expansion coefficient and specific heat of the lipid bilayer, respectively. Then, the relaxation strength of the ultrasonic properties Δ is described by

$$\Delta = \frac{\delta\kappa_m}{2\kappa_s} \tag{19}$$

in which κ_s denotes the compressibility of the aqueous solvent.

This theory is constructed on the assumption that the ultrasonic waves are coupled to the order parameter only through the thermal properties (entropy) and certainly predicts the slowing down of the ultrasonic relaxation time. This coupling mechanism has to exist in any physical system near the transition temperature, because the entropy necessarily varies according to the change in the order parameter. In this case, however, the coupling seems rather weak, because it is deduced theoretically that the relaxation strength is only weakly dependent on the temperature, as per Eqn. 18.

However, the experimental result in Fig. 6 indicates that the relaxation strength shows pronounced singularity in the vicinity of the transition temperature. The singularity of the relaxation strength appears even stronger than that of the relaxation time. This kind of singularity occurs when the ultrasonic property, that is, the compressibility, is directly related to the order parameter. For example, the order parameter of a gas-liquid transition is the density of the system [8]. Since the compressibility κ is generally proportional to the fluctuation of number density $\langle \delta\rho^2 \rangle$ [7,20],

$$\kappa = \frac{\langle \delta\rho^2 \rangle}{vT\rho^2} \tag{20}$$

the relaxation strength of the ultrasonic properties has to show the same temperature dependence as the order parameter in the case of the gas-liquid transition. In the same way, the anomaly in the relaxation strength in the lipid bilayer as well as

the analogy of π-A curves of lipid monolayer to P-V curves of gas-liquid systems strongly suggest direct coupling in the lipid bilayer. We suppose that the ultrasonic propagation is coupled to the membrane order through both thermal and mechanical properties.

Next, we have to examine the magnitude of the relaxation time. Several authors have reported various relaxations of dipalmitoylphosphatidylcholine membranes. The rotational relaxation time of hydrocarbon chains or probe molecules is about 1 ns [22]. The temperature jump technique has revealed two kinds of relaxation time in the range of milliseconds, which also show slowing down in the vicinity of the transition temperature [35]. Therefore, we have to discuss whether the ultrasonic relaxation time in Fig. 5 really corresponds to the relaxation of the order parameter. Since the order parameter is defined as an average value in semimicroscopic volume, the relaxations from molecular aspects do not correspond in principle to the relaxation of the order parameter. The rotational relaxation time of a lipid molecule is eliminated for this reason. However, the relaxation time of the order parameter fluctuation should not be very different from the motional relaxation time of a single molecule except for the immediate vicinity of the transition temperature, because the coherent length of the order parameter fluctuation normally does not exceed a few nanometers. Eqn. 14 indicates that the correlation length, ξ, is of the same order of magnitude as the molecular dimension, ξ_0, when $T - T^*$ is not very small. The relaxation time observed by the temperature jump technique appears too long as compared to the rotational relaxation time of hydrocarbon chains. In contrast, the relaxation time of several tens of nanoseconds is larger than the rotational relaxation time only by an order of magnitude and reasonable for the relaxation of the order parameter.

The comparison to the nematics also suggests that the ultrasonic relaxation corresponds to the fluctuation of the order parameter of the lipid bilayer. For example, N-(p-methoxybenziliden)-p-butylaniline (MBBA) is a rod-like molecule about 2 nm long [25]. It is established that the ultrasonic relaxation time of tens of nanoseconds represents the fluctuation of the orientational order of MBBA [26]. As discussed previously [9], hydrocarbon chains of phospholipids are similar to MBBA in their molecular dimension as well as in the structural transformation involved in the phase transition [9]. Therefore, it should be reasonable to consider that the ultrasonic relaxation in our sample is due to the fluctuation of membrane order. In consequence, the present results of the ultrasonic anomalies should indicate the enhancement of the fluctuation of membrane order as it is predicted by the Landau theory.

As is well known, it is very important and informative to determine the order of phase transitions, because many important features of physical properties may be derived from that order. In a first-order transition volume and entropy change discontinuously, while a second-order phase transition is characterized by the discontinuity or divergence of specific heat and compressibility. However, we have refrained from the discussion of the order of the gel-to-liquid crystal transition in this paper. We suppose that the sonicated vesicles 30 nm in diameter is too small a system in which to discuss the order of the transition, although the ultrasonic anomalies apparently indicate the second-order character. When the size of a system becomes smaller, thermal fluctuation of the system predominates and the phase transition is necessarily broadened. A single vesicle of about 30 nm in diameter contains only some thousands of molecules. This number is nearly the same as the number of particles assumed in usual computer simulation, which is considered to be too small for the study of transition behavior. In fact, the transition of multilamellar large vesicles (liposomes) is much sharper than the sonicated small vesicles [9,36]. Therefore, we have to measure the ultrasonic behavior of multilamellar liposomes in order to determine the order of the gel-to-liquid crystal transition. When we plot the relaxation time in Fig. 5 as a function of temperature in a double-logarithmic scale, we obtain an apparent critical exponent of about 0.2. However, we do not believe that the critical exponent of the lipid bilayer is 0.2. It is probably due to the small size of the system of sonicated vesicles. We conclude here only that the relaxation time and the magnitude of the order parameter fluctuation are anomalously enhanced in the vicinity of the transition temperature due to

the second-order character of this transition.

We realize that the present results apparently contradict other data: the shape of the transitional anomaly and the transition temperature. It seems helpful to discuss the temperature dispersion of the ultrasonic velocity in the high-frequency limit in order to clarify the relationship between the ultrasonic measurements and many data of molecular dynamics. The ultrasonic velocity of lipid bilayers in the gigahertz region was reported by LePesant et al. [37] using a Brillouin light scattering. A lipid multilayer containing a small amount of water was used as the sample in their experiment. Assuming that the concentration of their sample is approx. 1 g/cm^3, we can estimate the limiting velocity number from their data. The dotted line in Fig. 4 represents the recalculated data of the Brillouin scattering. Rather good agreement between the high-frequency limit of the present analysis and the data of LePesant et al. was obtained in the vicinity of the transition temperature. Since the order parameter is frozen at high frequencies, the mechanical properties are determined only by the average structure, i.e., average order parameter, which is independent of the magnitude of the fluctuation. Therefore, the ultrasonic velocity at the high-frequency limit does not show any anomaly due to the pronounced fluctuation of the order parameter.

The mobility of molecules in membrane measured by fluorescence or magnetic resonance shows only an abrupt sigmoidal change, and no anomalous dip or peak was found near the transition temperature [22,23,38]. This is probably due to the frequency range of the molecular motion. The rotational relaxation time of lipid-like molecules is about 1 ns, which is much shorter than the relaxation time of the order parameter. Therefore, if we regard the molecular motion as a probe of the order parameter fluctuation, it provides information only about the high-frequency limit. Thus, the temperature dispersion of the ultrasonic velocity at high-frequency limit is similar to the temperature dependence of various data of the molecular dynamics as well as the average order parameter [22,23,30,38].

The transition temperature determined in this work is different from the temperature determined by other techniques such as fluorescence measurement [22,23]. Previous studies of sonicated dipalmitoylphosphatidylcholine vesicles by fluorescence techniques provided a transition temperature of 37–39°C, while the present work revealed a transition temperature of 41.5°C. This apparent disagreement is due simply to the definition of the transition temperature. Fluorescence anisotropy shows a sigmoidal decrease in the vicinity of the transition point, and the midpoint of the sigmoidal curve is usually regarded as the transition temperature. It seems that this definition is introduced without consideration of the nature of the phase transition. This practical definition fails in general when critical phenomena predominate. For example, the order parameter of ferromagnets gradually decreases with increasing temperature, until the long-range order disappears at the Curie point. If we take the midpoint for the magnetic transition temperature, it will be very different from the real transition temperature, the Curie temperature. The same discussion applies to the lipid bilayer, because marked critical fluctuation is proved in this work in sonicated vesicles. In a phase transition which is very near a second-order transition, the change in the order parameter occurs rather smoothly over a wide temperature range. In such a case, the midpoint of a sigmoid does not necessarily coincide with the true transition temperature. Phenomenologically, the transition temperature may be defined by the divergent maximum of the second derivative of free energy: specific heat, compressibility and so on. In the present work, the maxima of the relaxation time, the relaxation strength, the ultrasonic absorption and the compressibility estimated from the ultrasonic velocity coincide with each other, indicating a transition temperature of 41.5°C. Comparing our results with other data, we propose that the transition temperature should be defined by the high-temperature end of various sigmoidal changes which probably corresponds to the appearance point of the membrane order.

Finally, we consider the biological significance of the relaxation of the membrane order, which is anomalously enhanced in the vicinity of the gel-to-liquid crystal transition. It is well known that membranes of some micro-organisms have a phase transition near the growth temperature [39,40]. The correlation between the phase transition and

the change in the growth rate or membrane permeability has been pointed out in relation to the biological significance of the phase transition [41,42]. However, the physical mechanism of the effect of the transition on the physiological functions has not yet been elucidated.

The plausible significance of the critical phenomena lies in the anomalies of passive permeability of membranes. Some small molecules have been found to permeate through membranes very quickly near the transition temperature [43,44]. As discussed previously, the anomalous increase of passive transport of membrane near the transition temperature is probably due to the enhanced fluctuation in membrane structure [11,12]. The probability of occurrence of large pores in the lipid bilayer is expected to increase when the fluctuation in the order of hydrocarbon chains is enhanced. This fact, together with the enhancement of the life-time of the pores which is suggested from the present work, will cause a pronounced increase in the permeability of small molecules through membranes. If the permeability of biological membranes really show this kind of permeability anomaly, it will have profound influence on the metabolism of a cell.

The present study of the ultrasonic relaxation also provides a new insight into the interaction between the lipid matrix and membrane proteins. The lipid matrix has to behave in different ways towards changes in protein structure at different speeds, particularly in the vicinity of the lipid-bilayer transition. When the structural change is much faster than the relaxation time of the lipid bilayer, the lipid bilayer behaves as a hard matrix. On the other hand, the lipid bilayer should be softer against slow structural change of proteins. When the temperature approaches the transition point, the relaxation strength increases abruptly. Thus, the nearer the temperature approaches the transition point, the softer the lipid matrix becomes towards the structural change slower than 1 μs. Therefore, if there is any membrane protein which requires for its function the movement of the lipid/protein interface, the relaxation of membrane order has to play a very important role in terms of hardness of the lipid matrix.

We may estimate the relaxation strength, $\delta\kappa_m$, of the compressibility of membrane from Δ by Eqn. 19. Table I shows the relaxation strength of the compressibility. It is about $0.2 \cdot 10^{-11}$ cm^2/dyn below 30°C as well as above 44°C and shows a maximum of about $1.1 \cdot 10^{-11}$ cm^2/dyn at the transition point. The absolute value of the compressibility of membrane was also calculated according to the method of analysis described previously [20] using the following equation:

$$[V] = -\frac{1}{2}\left(\frac{\kappa_m - \kappa_s}{\kappa_s} + \frac{\rho_m - \rho_s}{\rho_s}\right)\left(\frac{1+\delta}{\rho_m}\right) \qquad (23)$$

Assuming that the amount of hydrated water δ is about 0.4 g/g dry membrane and that $\rho_m \simeq 1.1$ g/cm^3, the absolute value of the compressibility at the high-frequency limit of dipalmitoylphosphatidylcholine membrane, was calculated to be from $3.3 \cdot 10^{-11}$ to $4.0 \cdot 10^{-11}$ cm^2/dyn throughout the temperature range studied. Hence, the relaxation strength is as large as 25% of the compressibility at the transition temperature. This change in the mechanical property appears to be large enough to affect the protein activities. Thus, not only passive but also active functions of biological membranes may be influenced by the pronounced relaxation of the lipid matrix in the vicinity of the gel-to-liquid crystal transition.

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