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ULTRASONIC STUDIES OF LIPID BILAYER. PHASE TRANSITION IN SYNTHETIC PHOSPHATIDYLCHOLINE LIPOSOMES

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The ultrasonic velocity at 3 MHz and the density in the nonsonicated and sonicated liposomes of dipalmitoylphosphatidylcholine have been measured in the temperature range from 0°C to 55°C. The results indicate that nonsonicated multilamellar vesicles undergo a weak first order transition which is analogous to the nematic-isotropic transition of liquid crystals. A sharp change in the ultrasonic velocity associated with the first order transition disappears when the multilamellar vesicles are sonicated. The bulk modulus of the lipid bilayer calculated from the ultrasonic velocity and the density of sonicated liposomes has a value of 3.0×10^{10} dyne/cm^2 at 20°C, reaches a minimum value of 2.1×10^{10} dyne/cm^2 at its transition temperature and increases slightly to 2.2×10^{10} dyne/cm^2 at 50°C.

1. Introduction

There is much interest in the physical properties of lipid bilayers, because they form a basic structure of biological membranes [1] and also because they are regarded as a lyotropic liquid crystal [2]. In particular a variety of physical techniques have been applied to the study of the phase transition in the lipid bilayers [3,4]. It has already been established that this transition is a gel-to-liquid-crystal transition and involves the melting of the hydrocarbon chains [5]. The lipid bilayer exhibits orientational order of hydrocarbon chains below the transition temperature T_c, and they are random in the high temperature liquid crystalline phase.

In spite of large amount of works, however, the physical characteristics of this phase transition are not necessarily clear; the order of the transition and the occurrence of the critical phenomenon is not confirmed. It has been reported that ESR spectra [6], polarization of fluorescence [6], X-ray diffraction spacing [8], density [9] and thermal properties [10] change in a very narrow temperature range in the nonsonicated multilamellar liposomes of DPPC and DMPC, suggesting a first order phase transition in the membrane [11]. On the other hand, permeability anomaly in the vicinity of T_c presumably indicates a critical phenomenon which is a distinctive feature of the second order phase transition [12,13]. Nagle has suggested based upon his model calculation and a density measurement of the DPPC lamellas that the transition is a 3/2-order transition [9]. In addition to such confusion about the characteristics of the phase transition in the lipid multilayers, it has been pointed out that a sharp change in the fluorescence, density and thermal properties disappears when the multilamellar liposomes are sonicated to form small single lamellar vesicles [14,15]. Thus, there is a paucity of accurate data in the transition regions.

This paper describes an attempt to elucidate the dynamical structures of the membrane in the vicinity of T_c by the ultrasonic measurements which has been a very powerful means for studying phase transitions and critical phenomena in various systems [16]. Recently Eggers and Funck have measured the ultrasonic attenuation in suspensions of small lipid vesicles and found an anomalous increase of the attenuation in the vicinity of T_c [17]. However, they measured the sound attenuation at only a few points near the transition temperature. In this work, we have measured

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the ultrasonic velocity at 3 MHz very accurately in the nonsonicated as well as sonicated liposomes with special attention to the transition regions. These studies indicate that the nonsonicated multilamellar liposomes undergo a weak first order transition analogous to the nematic-isotropic transition of liquid crystals, in which a critical phenomenon is accompanied by a small first order character. And it appears that in the sonicated vesicles there is also a sharp minimum of the ultrasonic velocity at T_c associated with a critical phenomenon but there is not an abrupt change due to the first order transition. The bulk modulus was first determined for the sonicated vesicles and was $2 \sim 3 \times 10^{10}$ dyne/cm^2 in the temperature range from 20°C to 50°C.

2. Materials and methods

2.1. Chemical substances

Synthetic L-α-dipalmitoylphosphatidylcholine (DPPC) and L-α-dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma Chem. Co. The powder of lipids was stored at about −25°C under nitrogen gas in order to prevent autooxidation. Lipids which showed one spot by thin layer chromatography on silica gel plates with a chloroform-methanol-acetic acid-water (25 : 15 : 4 : 2) mixture were used without further purification.

2.2. Preparation of liposomes

Lipids were dissolved in chloroform just before the measurement with a concentration of about 2 mg/ml. The solution was dried in a round-bottomed flask by a rotary evaporator. After adding the medium, 150 mM NaCl with a potassium phosphate buffer of pH 7.0, the flask was shaken by a vortex mixer for five minutes at about 50°C. Then, we obtained a turbid suspension of multilamellar liposomes with a diameter of a few micrometer. Small vesicles were prepared by sonicating it for fifteen minutes with an ultrasonic generator manufactured by Cho-onpa Industry Co. at 10 kHz and at a power level of 150 W in an atmosphere of nitrogen gas. Initially the temperature was 20°C, but during sonication the temperature of the sample rose above 50°C. Vesicles prepared by this method do not sediment during experiments of about fifteen hours. The weight concentration was determined after the measurement by drying the sample at 110°C in vacuum for a day.

2.3. Measurements of ultrasonic velocity and density

The ultrasonic velocity was measured at 3 MHz by a differential ultrasonic velocimeter which has been previously described [18]. In this apparatus, the difference in the ultrasonic velocity is directly measured between a sample and the medium in twin cells. Accuracy of the difference in the ultrasonic velocity is about ±0.7 cm/s and the long-term stability is ±0.7 cm/s/day. It should be noted that the use of this apparatus has permitted us to measure the ultrasonic velocity of small amount of dilute liposome suspensions with sufficiently high accuracy.

A vibrational densitometer [19] was constructed for the study of the temperature dependence in the density of liquids. The principle of the apparatus is to measure the period of vibration of a U-shaped calipary tube in which a sample liquid is charged. We have carefully calibrated the apparatus constants against the temperature and obtained the following relation between the density ρ and the period T of vibration in a temperature region from 20°C to 45°C.

$$T - T_0 = a(\rho - \rho_0) + b + c\theta, \qquad (1)$$

in which a, b and c are constants and θ is the temperature. Here, the period T and the density ρ correspond to the sample, while T_0 and ρ_0 are the period and the density of the medium. Since the last term $c\theta$ in eq. (1) was independent of the sample density, it appeared to be due to the temperature dependence of some characteristics of the vibrating capilary or its driving ciucuits. The apparatus and the calibration of eq. (1) will be described in detail elsewhere.

The temperature of samples was controlled within ±0.001°C in a water bath, and all measurements were performed after equilibrating the temperature. About twenty minutes were allowed to establish equilibrium at a new temperature and it took about fifteen hours to complete an experiment. Although sonicated vesicles were very stable during the experiments for fifteen hours, the sedimentation rate of nonsonicated liposomes was so high that it was necessary to stirr the sample frequently. The sample liquid may not be

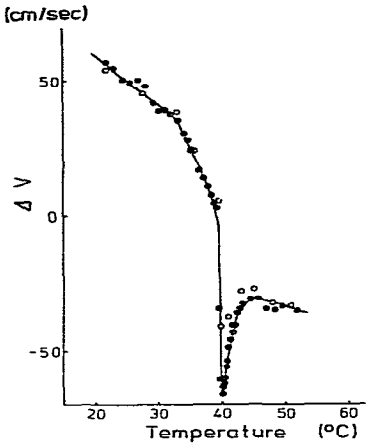

Fig. 1. Temperature dependence of the ultrasonic velocity at 3 MHz in nonsonicated liposomes of DPPC with the dry weight concentration of 2.63 mg/ml. All measurements were made in equilibrium. The closed circles represent heating run and the open circles are the cooling run.

stirred in a capilary tube of the vibrational densitometer, however, and we could not obtain a quantitatively reliable results on the temperature dependence of the density of nonsonicated liposomes.

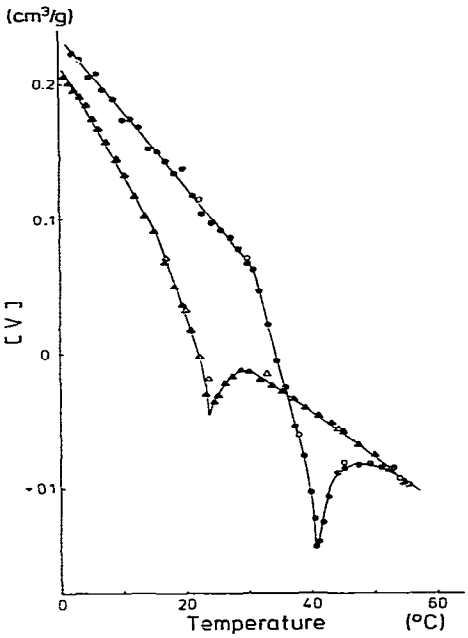

Fig. 2. Limiting velocity numbers of sonicated vesicles of DPPC (● ○) and DMPC (▲ △). The limiting numbers were calculated from the ultrasonic velocity in suspensions of sonicated DPPC vesicles with the dry weight concentration of 1.79 mg/ml and of DMPC vesicles with 2.74 mg/ml.

3. Results

The excess ultrasonic velocity ΔV of the nonsonicated multilamellar liposomes of DPPC with the dry weight concentration of 2.63 mg/ml was measured in the temperature range between 20 and 53°C. Fig. 1 represents the behaviour of ΔV, in which closed circles are a sequence of increasing temperatures and open circles are a sequence of decreasing temperatures. Distinct ultrasonic anomalies are found in the vicinity of the transition temperature T_c. A large and steep change in ΔV occurs at T_c, which indicates an abrupt structural change in the membrane. The width of the steep change is about 0.4°C, which is nearly equal to the reported values; 0.2°C as measured by calorimetry [10] and dilatometry [9]. In addition, anomalous dip of ΔV is distinct in a wide temperature range between 34°C and 50°C. This anomalous dip is very similar to the ultrasonic anomaly due to a critical phenomenon studied in such simple systems as a gas-liquid transition, a magnetic transition and so on [16]. The temperature dependence of ΔV changes its slope at 34°C, corresponding to the so-called pretransition of DPPC membrane [9,10]. The temperature dependence is reversible except for the immediate vicinity of T_c; ΔV is a little larger in the cooling run than in the heating run.

The sonicated vesicles of DPPC and DMPC were measured to study the effect of the hydrocarbon chain length. Fig. 2 shows the limiting velocity number $[V]$ of the DPPC and DMPC vesicles, which is defined by

$$[V] = \lim_{c \to 0} (V - V_0)/V_0 c, \qquad (2)$$

where V and V_0 are the ultrasonic velocity of the sample and the medium, respectively, and c is the dry

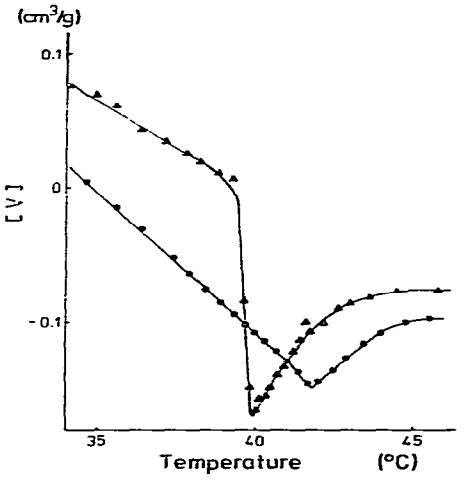

Fig. 3. Limiting velocity number of sonicated vesicles (•) is compared with that of nonsonicated multilamellar liposomes (▲) in the vicinity of the transition temperature.

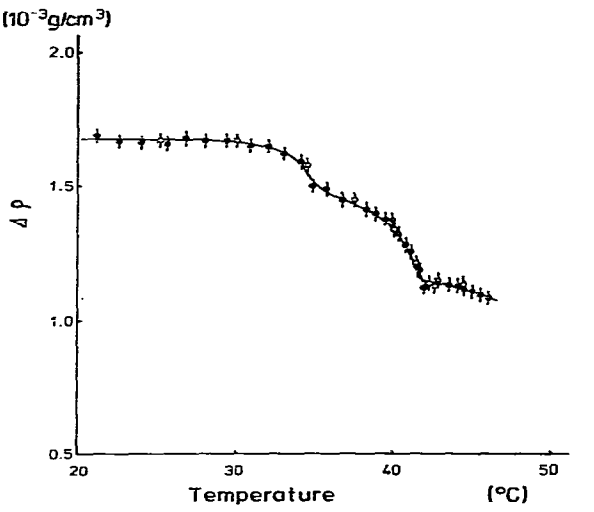

Fig. 4. Temperature dependence of the density of sonicated DPPC vesicles with the dry weight concentration of 13.0 mg/ml. The closed circles are a sequence of increasing temperatures and the open circles are a sequence of decreasing temperatures.

weight concentration [20]. The dry weight concentration of the samples was 1.79 mg/ml for DPPC and 2.74 mg/ml for DMPC. Since $(V - V_0)$ is a linear function of c in this concentration range, the limiting velocity number was calculated simply dividing $(V - V_0)/V_0$ by c. The sonicated vesicles of DPPC and DMPC resemble each other in the general features of the temperature dependence of $[V]$: the limiting velocity number decreases when the temperature is increased, and there is an anomalous dip in the vicinity of T_c. Furthermore, the limiting velocity number of these samples quantitatively agree in the temperature range far from T_c. The transition temperature of the sonicated vesicles of DPPC and DMPC was 42.0°C and 24.0°C, respectively, in accordance with previously reported values [10].

Apparently, the behaviour of the ultrasonic velocity in sonicated vesicles are different from that in nonsonicated liposomes. The nonsonicated and sonicated liposomes are compared quantitatively in fig. 3, in which the temperature region just above and below T_c is expanded. There is a sharp jump of $[V]$ as large as 0.16 cm³/g in the nonsonicated multilamellar liposomes, while the sonicated vesicles does not show such an abrupt change. The transition temperature of the nonsonicated multilamellar liposomes is higher than that of the sonicated vesicles by about two degrees. The quantitative difference is also evident: the limiting velocity number of the nonsonicated liposomes is larger than that of the sonicated vesicles except for the transition region.

We have also measured the density of the sonicated DPPC vesicles by the vibrational densitometer. Fig. 4 represents the temperature dependence of the excess density of the sonicated DPPC vesicles with a dry weight concentration of 13.0 mg/ml. The density changes abruptly just below the transition temperature of 42°C, and a small sigmoidal change of the density is observed at the pretransition of 34°C. The curve in fig. 4 qualitatively agrees with the data previously described by Sheetz and Chan [14]. If the critical phenomenon occurs in the vicinity of T_c, the temperature dependence of density has to obey some critical exponential form. In fig. 5, the density just below T_c is plotted against the temperature in a double logarithmic scale. It appears that there is a linear relation between $\log(\Delta\rho - \Delta\rho_c)$ and $\log(T_c - T)$ in the temperature range of $0°C < T_c - T < 8°C$, and the critical exponent is about 0.67

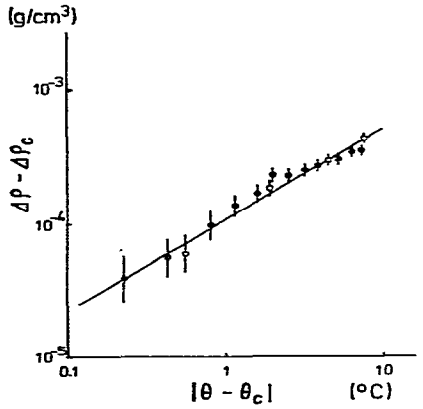

Fig. 5. Critical exponent of the density of sonicated DPPC vesicles.

± 0.07. Although the error is fairly large, fig. 5 suggests that a critical phenomenon occurs near the transition temperature of the sonicated DPPC vesicles.

The density measurement was carried out for the nonsonicated liposomes in the temperature range from 22°C and 45°C, and a sharp decrease in the density was found at T_c which is consistent with the ultrasonic behaviours. However, it has turned out that a sedimentation rate of multilamellar liposomes is too fast to measure the temperature dependence of the density without stirring a sample, that is difficult in the vibrational densitometer. Therefore, only the value at 22°C was reliable quantitatively.

4. Discussion

4.1. Physical characteristics of phase transition

In relation to the characteristics of the phase transition in the multilamellar DPPC liposomes, there are two remarkable features in the ultrasonic behaviour; a very sharp jump at T_c and an anomalous dip of the ultrasonic velocity in the temperature range, $|T - T_c| < 8$°C. The width of the sharp change in the ultrasonic velocity is comparable to those of thermal properties and density [9,10], and the anomalous dip is found in the same temperature regions as the permeability anomaly [12,13]. The ultrasonic velocity measurement in fig. 1 is the first experiment which has definitely shown these two kinds of transition anomalies simultaneously. It is known by the theoretical and experimental studies of phase transitions and critical phenomena that the anomalous dip in the ultrasonic velocity in the vicinity of T_c is due to a critical slowing down of the correlation time while the more abrupt change at T_c corresponds to a first order phase transition [16,21,22]. Therefore, the two kinds of ultrasonic anomalies probably indicate that the phase transition in the nonsonicated multilamellar DPPC liposomes is very similar to other critical phenomena but there is a small first-order character to the phase transition in the membranes: the transition in the DPPC bilayers is a weak first order phase transition.

At the onset of a critical phenomenon the affinity of the order parameter sharply decreases, which results in a larger fluctuation around an equilibrium state and to a longer relaxation time [21]. When the equilibrium state is periodically perturbed by ultrasonic waves, a phase-shift due to a critical slowing down of the relaxation time occurs between the oscillations of pressure or temperature and the equilibrium state of the system. This leads to an anomalous ultrasonic absorption and velocity dispersion. The ultrasonic velocity and absorption per wavelength is described for a single relaxation process as,

$$V^2 = V_0^2 + (V_\infty^2 - V_0^2)\frac{\omega^2\tau^2}{1+\omega^2\tau^2}, \tag{3}$$

$$\alpha\lambda = \frac{\pi(V_\infty^2 - V_0^2)}{V^2}\frac{\omega\tau}{1+\omega^2\tau^2} + \frac{\pi B}{V^2}\omega, \tag{4}$$

in which V, V_0 and V_∞ are the ultrasonic velocities at the angular frequencies of ω, zero and infinity, respectively. The second term of eq. (4) is a classical absorption of sound due to shear and bulk viscosities and thermal conductivity. When the frequency of sound is sufficiently low or high, the ultrasonic velocity is equal to V_0 or V_∞ and the ultrasonic attenuation arises only from the classical mechanisms. Since the enhanced isothermal compressibility causes the sharp decrease of V_0 in the vicinity of a critical point, the ultrasonic velocity at intermediate frequencies also shows an anomalous dip at T_c. The sound attenuation has a maximum value under the condition that $\omega\tau \sim 1$.

Thus, the ultrasonic anomalies in the vicinity of a critical point is due to the anomalous decrease of V_0 and increase of the relaxation time τ.

Recently Eggers and Funck have measured the ultrasonic attenuation in small vesicles of DMPC and found an anomalous increase of $\alpha\lambda$ [17]. Their results are consistent with the anomalous dip of the ultrasonic velocity in the present work, and it appears that the relaxation time near T_c is in the range of 0.001 and 0.1 μs Although Eggers and Funck have not attributed the anomalous ultrasonic attenuation to a critical phenomenon, these ultrasonic behaviours are reasonably explained by the critical slowing down in eqs. (3) and (4).

From a molecular aspect a critical phenomenon is characterized by the coexistence and the dynamical fluctuation of the ordered and disordered phases, while there occurs a discontinuous phase change between the two phases at a first order transition point [23]. It may be concluded, therefore, that in the multilamellar DPPC liposomes the gel and liquid crystalline phases coexist and fluctuate in the vicinity of the transition point at which a small discontinuous phase change takes place. For further discussions of the present data it seems helpful to compare the ultrasonic velocity in the lipid bilayers with that in the nematic liquid crystals, because both systems are formed by long molecules and the ultrasonic behaviours in the nematics have been well investigated [22,24,25]. Rod-like molecules are oriented in the same direction in a nematic liquid crystal and the directional order disappears through the nematic-isotropic transition. In the gel phase of the lipid bilayer hydrocarbon chains also have an orientational order which is partly lost in the liquid crystalline phase. Therefore, this comparison appears to be pertinent in spite of some contrasts between the lipid bilayers and the nematics: the order parameter is not so well defined in the lipid bilayers as in the nematics because of the high flexibility of hydrocarbon chains and the layer structures.

In fig. 6, the ultrasonic velocity at 3 MHz in the nonsonicated multilamellar liposomes is shown together with the results at 2 MHz of the nematic isotropic transition of p-azoxyanisole [25]. The scales of the ultrasonic velocity is adjusted so that the magnitude of the anomaly is comparable in the same diagram. In both systems the ultrasonic velocity

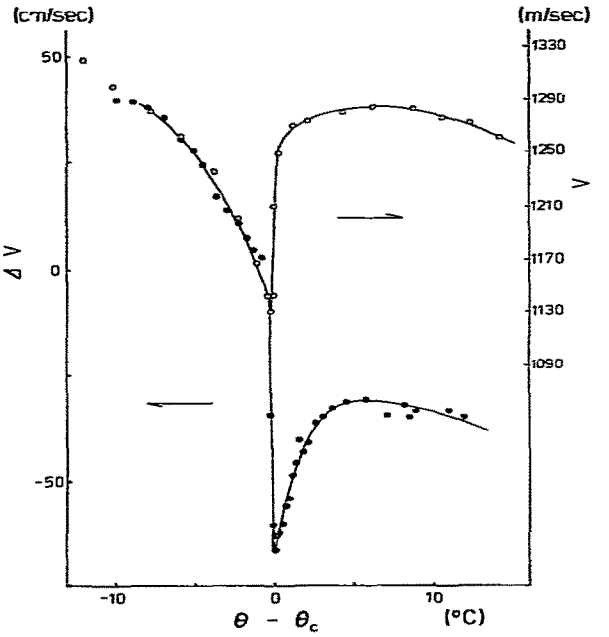

Fig. 6. Ultrasonic velocity near the transition temperature of nonsonicated DPPC liposomes (•) at 3 MHz and a nematic liquid crystal, p-azoxyanisole (○), at 2 MHz [25].

abruptly changes at T_c due to the first order character and anomalously decreases in the vicinity of T_c corresponding to the critical phenomenon. The qualitative features of these curves are the same except that the directions of the sharp changes are opposite, and we can assume that the weak first order transition in the lipid bilayer is established by this analogy.

The magnitude of the sharp change of about 70 cm/s in the lipid bilayer seems to be very small as compared with 120 m/s in p-azoxyanisole. But this quantitative difference is apparent and results from the small weight concentration of liposomes, 2.63 mg/ml. When the volume concentration c' is much smaller than unity, the magnitude of the sharp change in the ultrasonic velocity ΔV of the liposome suspension is approximately related with the change ΔV_m of the lipid bilayer membrane as,

$$\Delta V/V \approx (\Delta V_m/V_m)c', \tag{5}$$

where V and V_m are the ultrasonic velocities of the

liposome suspension and the lipid bilayer, respectively. The relation between the weight and volume concentrations is derived from the weight of wet membrane.

$$\rho_m c' = c(1 + \delta), \quad (6)$$

in which ρ_m is the density of wet membrane and δ is the weight of water hydrated to 1 gram of dry membrane. Since we may roughly assume that $(1 + \delta)/\rho_m \sim 1$ [20], the velocity change ΔV_m in the lipid bilayer is estimated through eqs. (5) and (6). This rough calculation gives the velocity change of 270 m/s in the lipid bilayer which is comparable to 120 m/s in p-azoxyanisole. Consequently, the phase transition of the lipid bilayer is analogous to the nematic-isotropic transition not only in the qualitative features but also in the magnitude of the first order character.

Concerning the difference in the direction of the sharp change, we cannot present any definite explanation, but a plausible interpretation is as follows. The hydrocarbon chains in the lipid bilayer are flexible, whereas the molecules in a nematic liquid crystals are considered to be rigid. Then, hydrocarbon chains in the liquid crystalline phase easily change their conformation relaxing an external stress. On the contrary, molecules of nematic liquid crystals do not deform by a stress, although there is a larger vacancy in the isotropic phase. Then it is reasonable that the increment of the bulk modulus from ordered to disordered phase is smaller in the lipid bilayer than in the nematic liquid crystals. Since the ultrasonic velocity is generally represented by

$$V = \sqrt{K/\rho}, \quad (7)$$

the increment of the ultrasonic velocity also has to be smaller in the lipid bilayer. We suppose this is why the ultrasonic velocity sharply decreases at T_c in the lipid bilayer while it increases in the nematic liquid crystals.

In contrast to the nonsonicated multilamellar liposomes, only an anomalous dip is found at the transition of the sonicated vesicles. The temperature dependence of the density was also more gentle than in the multilamellar liposomes, which is consistent with the results of Sheetz and Chan [14]. Furthermore, the density of the sonicated vesicles seems to follow the exponential form with a critical exponent of 0.67. These facts indicate that the phase transition in the sonicated vesicles is not a first order transition but probably a second order transition. Enhanced mobility of hydrocarbon chains in the gel phase has been reported in the sonicated vesicles and has been attributed to the distortion of the hydrocarbon chain packing, based upon the NMR and fluorescence measurements [7,14]. A distortion of hydrocarbon chains gives rise to the increase of the entropy per a chain, causing a decrease in the entropy difference ΔS between the gel and liquid crystalline phases. Since the latent heat Q of the transition is represented by [26]

$$Q = T_c \Delta S, \quad (8)$$

the decrease in ΔS results in the reduction of the latent heat. The disappearance of the sharp change in ΔV may be due to such effect of molecular distortion.

The temperature dependence of the sound velocity in the nonsonicated multilamellar liposomes shows only poor reversibility as far as the dip is concerned. This observation is in agreement with the previously reported hysterisis [27]. It is known that a first order transition may accompany a supercooling or superheating. Therefore, the poor reversibility may be due to the fact that the phase transition is of the first order in the multilamellar liposomes.

Suurkuusk et al. have reported that the transition temperature of sonicated vesicles is about 37°C and is lower than that of nonsonicated liposomes by 4°C. Our results by the ultrasonic velocity measurements seems to be inconsistent with their fluorescence depolarization measurements [15]. This inconsistency, however, probably results from the difference in the methods: the fluorescence depolarization reflects a rotational relaxation of a single molecule, while the ultrasonic velocity depends on the long range order of hydrocarbon chains. The difference between these methods is evident from the data in the vicinity of the transition temperature. An anomalous dip due to a critical phenomenon is not found by the fluorescence depolarization measurements. If we remove the anomalous dip from the temperature dependence of the ultrasonic velocity of DPPC liposomes in figs. 1 and 2, the middle point of the abrupt change becomes about 37°C in the sonicated vesicles, and it remains 40°C in the nonsonicated liposomes. The same explanation settles the inconsistency of the fluorescence and calorimetric measurements in the work of Suurkuusk et al. [15]. The critical phenomenon is characterized by a large

fluctuation around the equilibrium state, which is essentially a cooperative phenomenon. Thus, it has been difficult to prove the critical phenomenon in the membrane by the measurements of fluorescence, ESR and NMR which reflect the mobility of a single molecule.

On the other hand, light scattering measurements may provide very singular results due to critical opalescence. In fact, Abramson has measured a maximum at T_c of 90°-light scattering in the sonicated DPPC vesicles [28]. However, this maximum was very small presumably because the size of vesicles were much smaller than the wave length of the light: the correlation length of the fluctuation is limited within the vesicle size. The concept of a critical phenomenon leads us to the prediction that the light scattering intensity of large liposomes will show a sharp maximum at T_c due to the critical opalescence if the high turbidity of nonsonicated liposomes is adequately corrected.

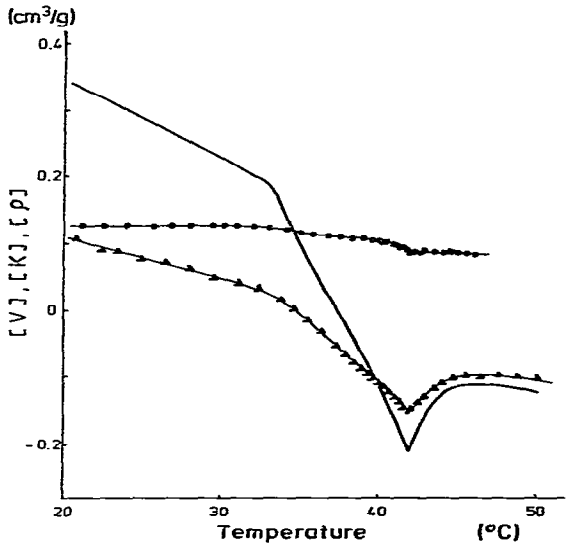

Fig. 7. Limiting numbers of velocity (▲), density (●) and bulk modulus (—) of sonicated DPPC vesicles.

4.2. Bulk modulus and density of lipid bilayer

We have determined the bulk modulus of a membrane that is a very useful quantity in relation to the barrier properties. For instance, the incorporation of cholesterol into the lipid bilayer causes a remarkable increase of the bulk modulus which will be reported elsewhere. The following relation between the limiting numbers of velocity $[V]$, density $[\rho]$ and bulk modulus $[K]$ is used for the calculation of the bulk modulus K_m and the density ρ_m of the lipid bilayer

$$[V] = \tfrac{1}{2}([K] - [\rho]). \tag{9}$$

Here, $[K]$ and $[\rho]$ are described by the following equations.

$$[K] = \lim_{c\to 0} \frac{K - K_0}{K_0 c} = \frac{K_m - K_0}{K_m} \frac{1+\delta}{\rho_m}, \tag{10}$$

$$[\rho] = \lim_{c\to 0} \frac{\rho - \rho_0}{\rho_0 c} = \frac{\rho_m - \rho_0}{\rho_0} \frac{1+\delta}{\rho_m}, \tag{11}$$

in which K_0 and ρ_0 are the bulk modulus and the density of the medium, respectively. Equations (10) and (11) have been derived from the combination of eq. (6) and the additivity of the density ρ and the compressibility $1/K$ which has been proved by a theory of sound propagation in a spherical cell suspension [29].

The temperature dependence of $[K]$ calculated by eq. (9) is shown in fig. 7 together with $[V]$ and $[\rho]$ for the case of the sonicated DPPC liposomes. It is manifest that the anomaly of the bulk modulus is the cause of the dip in the ultrasonic velocity. To calculate the bulk modulus K_m and the density ρ_m of the lipid bilayer from $[K]$ and $[\rho]$ in fig. 7, it is necessary to assume the amount of hydration. The adsorption experiment has revealed the hydrated water of 0.44 ~ 0.48 gram per one gram of egg yalk phosphatidylcholine [30]. Similar value of the hydration of membranes has been obtained by the ultrasonic and density measurements [29]. Therefore, it seems reasonable to assume the hydration of 0.5 gram water per one gram of the DPPC lipid bilayer. Table 1 shows the density and the bulk modulus of the lipid bilayer in the sonicated and nonsonicated DPPC liposomes which are calculated on the assumption that $\delta = 0.5$ and 0.3. The calculated values for the case of $\delta = 0.3$ are listed in order to show that the variation in the hydration does not affect the results. The density of the sonicated DPPC membrane is about 1.10 g/cm^3 below T_c and 1.05 ~ 1.06 g/cm^3 above T_c. The change in the

Table 1
The density and the bulk modulus of the lipid bilayer in sonicated and nonsonicated liposomes

Temperature	Density (g/cm^3)			Bulk modulus ($\times 10^{10}$ dyne/cm^2)		
	$\delta = 0.5$	$\delta = 0.3$	Medium	$\delta = 0.5$	$\delta = 0.3$	Medium
(Sonicated)						
20	1.099	1.115	1.0049	3.01	3.19	2.24
30	1.096	1.112	1.0021	2.77	2.87	2.31
42	1.060	1.070	0.9978	2.07	2.02	2.37
50	1.048	1.056	0.9947	2.20	2.17	2.39
(Nonsonicated)						
22	1.081	1.094	1.0045	3.15	3.37	2.25

density is $4 \sim 5\%$ of the value of the density, which is consistent with the earlier experiments [9,14]. The bulk modulus of the lipid bilayer is first determined in this work and turned out to be about 3.0×10^{10} dyne/cm^2 below T_c and 2.2×10^{10} dyne/cm^2 above T_c, that is, the bulk modulus changes by about 30%. The bulk modulus of the DPPC membrane above T_c is even smaller than that of the medium. It is considered that the lipid bilayers provide the structural framework of the membrane, and the small value of the bulk modulus in the liquid crystalline phase is remarkable in relation to the suggested importance of the membrane fluidity. The bulk modulus determined by the ultrasonic studies will serve as an useful measure of the properties of a membrane as a barrier.

4.3. Physiological meaning of critical phenomena

We have shown that a distinct critical phenomenon occurs in the temperature range of $|T - T_c| < 8°C$ for both the multilamellar and sonicated liposomes. In general, characteristic phenomena near the critical point are summarized as follows [12]:

(1) The fluctuation of the order parameter anomalously increases, when the temperature approaches the transition point.

(2) The relaxation time increases anomalously.

(3) The correlation length of the order parameter increases in the vicinity of T_c.

Although the order parameter in the lipid bilayer is not so well defined as in the nematic liquid crystals, we may regard the order parameter as an effective measure of the ordering of hydrocarbon chains; the orientational order and the hexagonal packing. Then, we may present the following dynamical structure of the lipid bilayer in the vicinity of T_c based upon the general characteristics of the critical phenomena. There are domains of ordered and disordered lipid bilayer. In the ordered domain, hydrocarbon chains are in the all-trans conformation and hexagonally packed. In the disordered domain, chains are moving rapidly and contain many gauche bonds. The domains are not statically formed, but dynamically fluctuate with the relaxation time of $0.001 \sim 0.1$ μs. The magnitude of the fluctuation, the size of domains and the relaxation time become larger when the temperature approaches the transition point.

The picture of a fluctuating membrane leads us to the idea of two kinds of transition effects on membrane functions. One is the effect on the activity of membrane enzymes and the other is the effect on the basic membra function as a barrier. With respect to the former effect, only a discontinuous enzyme activity at T_c has been shown for several enzymes, which is explained by a change in the membrane fluidity from the gel to liquid crystalline phases [31,32]. Thus, we have no evidence of the anomalous behaviour of enzyme activity, and enzymes may be insensitive to the fluctuation of hydrocarbon chain ordering.

An anomalous increase in permeability is reported recently for several membranes. The lipid bilayer of DPPC, dipalmitoylphosphatidylglycerol and DMPC shows an enhanced permeability to Na^+, sucrose and a spin probe [12,13]. Linden et al. have also measured an anomalous increase of sugar transport through escherichia coli membrane [33]. These observations well

correlate with our results; an anomalous decrease of the bulk modulus. Some attempts have been made to explain this phenomenon by the increase in the length of the phase boundary between the ordered and the disordered phases [13]. But the magnitude of the fluctuation, the correlation length and the relaxation time simultaneously increase in the critical region, as mentioned above. Therefore, the permeability anomaly is not necessarily due to the size of domains. We suppose rather that it is caused by the critical slowing down, because the permeation process is a dynamical process. At any rate, the permeability anomaly is due to the critical phenomenon and is very suggestive in connection with the fact that the transition temperature is found near the growth temperature in some bacterial membranes.

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