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Ultrasonic Studies of Lipid Bilayer Phase Transition†

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The ultrasonic velocity and absorption of nonsonicated liposomes of dipalmitoyl phosphatidylcholine were measured as a function of the temperature. The ultrasonic velocity showed a broad anomalous dip together with very sharp change at the transition temperature. The ultrasonic absorption was enhanced anomalously in the same temperature range as the dip of the velocity. These transition behaviors indicate that dipalmitoyl phosphatidylcholine bilayer undergoes a weak first order transition in which the transition is of first order but very near the second order transition. It is suggested in comparison to the monolayer phase diagram that the phase transition of dipalmitoyl phosphatidylcholine bilayer occurs a little below the critical point of monolayers.

INTRODUCTION

Ultrasonic techniques have been applied to lipid bilayers by several investigators.[1-5] The mechanical relaxation,[2,5] the anisotropy of mechanical properties[4] and the transition properties[3] of lipid bilayers were studied by these works. However, systematic studies of the frequency dispersion as well as temperature dependence of the ultrasonic velocity and absorption in the vicinity of the transition point have little been carried out because of several technical difficulties; very large temperature coefficient of the ultrasonic velocity in water and the necessity of large amount of samples at low frequency range.

In the present work, we simultaneously measured the ultrasonic velocity and absorption for the first time through the phase transition of dipalmitoyl phosphatidylcholine liposomes by use of a differential apparatus.[6] Anoma-

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lous decrease of the ultrasonic velocity and enhancement of the absorption which were observed in the vicinity of the transition temperature indicated a distinct critical phenomenon in the lipid bilayer structure.[3,7] Whereas the sharp change of the velocity at the transition temperature showed that the transition is of first order. Since the transition behaviors of lipid bilayers has to arise from the state of each lipid monolayers, we explained these anomalies in terms of the pressure-area phase diagram of lipid monolayers.[8,9]

METHODS AND MATERIALS

Ultrasonic velocity and absorption were measured by a differential type apparatus at 3 MHz, which was originally designed for the measurement of the ultrasonic velocity[6] and improved for the ultrasonic absorption measurement. The details of the principle as well as the performance of the apparatus in the measurement of the ultrasonic velocity have been reported in Ref. 6. The ultrasonic pulse was triggered by the second echo of the preceding pulse. The period T of successive pulse gives the ultrasonic velocity V as

$$T = T_e + 4L/V, \tag{1}$$

where T_e and L are the electric delay time and the effective cell length. In order to derive the information about suspended lipid bilayers, we determined the difference in the ultrasonic velocity ΔV between a liposomal suspension and solvent using two cells with the same cell length.

The excess ultrasonic absorption $\Delta\alpha$ was determined by monitoring the signal amplitude with a synchroscope,

$$\Delta\alpha = \frac{1}{4L} \ln \frac{E_0}{E}, \tag{2}$$

in which E and E_0 are the amplitude of the second echo for the sample and the solvent, respectively. The accuracy was ± 0.7 cm/sec for the velocity and $\pm 10^{-3}$ cm^{-1} for the absorption.

Dipalmitoyl phosphatidylcholine was purchased from Sigma Chem. Co. and used without further purification. Nonsonicated liposomes were prepared according to the method described previously.[3] Aqueous solution of 150 mM NaCl and pH 7.0 was used for the solvent. The diameter of liposomes was from submicrometer to about ten micrometer as observed by polarized optical microscope.

RESULTS AND DISCUSSIONS

The excess ultrasonic velocity and absorption of nonsonicated dipalmitoyl phosphatidylcholine liposomes of 2.7 mg/ml were measured at 3 MHz in the

temperature range between 10 and 55°C, as shown in Figure 1. As the temperature was raised, the excess ultrasonic velocity decreased from 60 cm/sec at 15°C to −40 cm/sec at 55°C through a small break point at 34°C as well as a deep minimum point at 42°C. The ultrasonic absorption showed a broad anomalous peak at 42°C corresponding to the anomalous dip of the velocity. It should be noted also that the ultrasonic velocity decreases very sharply at the transition temperature of 42°C.

The transition temperature of 42°C coincides with the temperature of a gel to liquid crystal transition which was measured by a variety of techniques.[10-14] Below this transition temperature hydrocarbon chains are oriented perpendicular to the plane of the lipid bilayer and form a two-dimensional hexagonal lattice. These orientational as well as positional order of hydrocarbon chains melt above the gel to liquid crystal transition. The present results indicate that the gel to liquid crystal transition causes a very sharp change as well as a broad anomalies of the ultrasonic velocity and absorption.

A small break point at 34°C corresponds to the pretransition which was studied by calorimetry, X-ray diffraction and electron spin resonance.[13-16] It is reported that the pretransition is related to the intermembrane ordering,[15,16] but the molecular mechanism of this transition is not yet clear.

It appears that the very sharp change at the gel to liquid crystal transition indicate the first order nature of this transition. In our measurement, there are several factors which may broaden this sharp change. First, our sample lipid may have some contamination less than 1%, although thin layer chromatography showed a single spot. Second, small amount of single lamellar small vesicles which does not show any discontinuous sharp change[3] may be contained in the suspension. Third, the accuracy is not sufficiently good, when we measure very dilute suspensions. However, considering these factors, the sharpness of the velocity decrease at the transition point is remarkable and almost comparable to that of the nematic-isotropic transition which is known to be a weak first order transition.[3,17] This fact strongly suggests that the lipid bilayer of dipalmitoyl phosphatidylcholine also undergoes a first order transition. Similar result is obtained by Albon and Sturtevant who performed a thermal analysis of the gel to liquid crystal transition using very pure dipalmitoyl phosphatidylcholine and showed that the width of the endothermic peak is due to the impurity.[18]

On the other hand, the broad anomalies seem to indicate that there occurs a marked critical phenomenon in the temperature range from 34 to 50°C. In general, the restoring force against the fluctuation of an order parameter from the equilibrium value gradually vanishes when the temperature approaches a second order phase transition point. Then, the structural fluctuation increases anomalously, resulting in the softening of the system and the slowing down of the relaxation time.[19] This phenomenon is called a critical phenomenon. Although the transition of the dipalmitoyl phosphatidylcholine bilayer is of first

order, the broad anomalous behaviors of the ultrasonic velocity and absorption suggest that the transition is very near the second order transition which is characterized by the critical phenomenon.

Let us assume a single relaxation time τ of the order parameter. Then, the ultrasonic propagation constants are described by,[20]

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

$$\alpha = \frac{V_\infty^2 - V_0^2}{2V^3} \cdot \frac{\omega^2\tau}{1 + \omega^2\tau^2}, \tag{4}$$

where V_∞ and V_0 are the ultrasonic velocity at sufficiently low and high frequencies as compared to the relaxation frequency. The relaxation time of $10^{-9} \sim 10^{-8}$ sec is reported by Eggers *et al.* and Gamble *et al.* for dipalmitoyl phosphatidylcholine bilayers, which was measured by the ultrasonic absorption.[2,5] This value of the relaxation time is in good agreement with the rotational relaxation time of hydrocarbon chains. Therefore, $\omega\tau$ is expected to be smaller than unity at the frequency of 3 MHz, and Eqs. (3) and (4) are approximated by

$$V^2 \simeq V_0^2 = \frac{1}{\rho\kappa_0}, \tag{5}$$

$$\alpha \simeq \frac{V_\infty^2 - V_0^2}{2V^3}\omega^2\tau, \tag{6}$$

in which ρ and κ_0 are the density and the compressibility at low frequencies, respectively. The broad anomalous dip of the ultrasonic velocity in Figure 1 combined with Eq. (5) indicates that the compressibility of the lipid bilayer is enhanced in the transition regions (softening of the lipid bilayer). In addition, the anomalous peak of the absorption together with Eq. (6) indicate that the relaxation time is also enhanced (critical slowing down). Kawato *et al.* actually measured a small but anomalous peak of the rotational relaxation time of fluorescent probes in the vicinity of the transition temperature.[11]

Consequently, the dipalmitoyl phosphatidylcholine bilayer undergoes a weak first order transition in which the transition is of first order but very near the second order transition. Namely, the structural fluctuation of lipid bilayer is considerably large in the wide temperature range between 34 and 50°C as in the second order transition, but the latent heat is observed in the same sample due to the first order character of the transition.[18]

It is difficult to derive these transition properties from microscopic viewpoint. However, a phenomenological comparison of the present result to the lateral pressure-area phase diagram of lipid monolayer seems to be helpful for the understanding of the lipid bilayer transition, because it is considered that

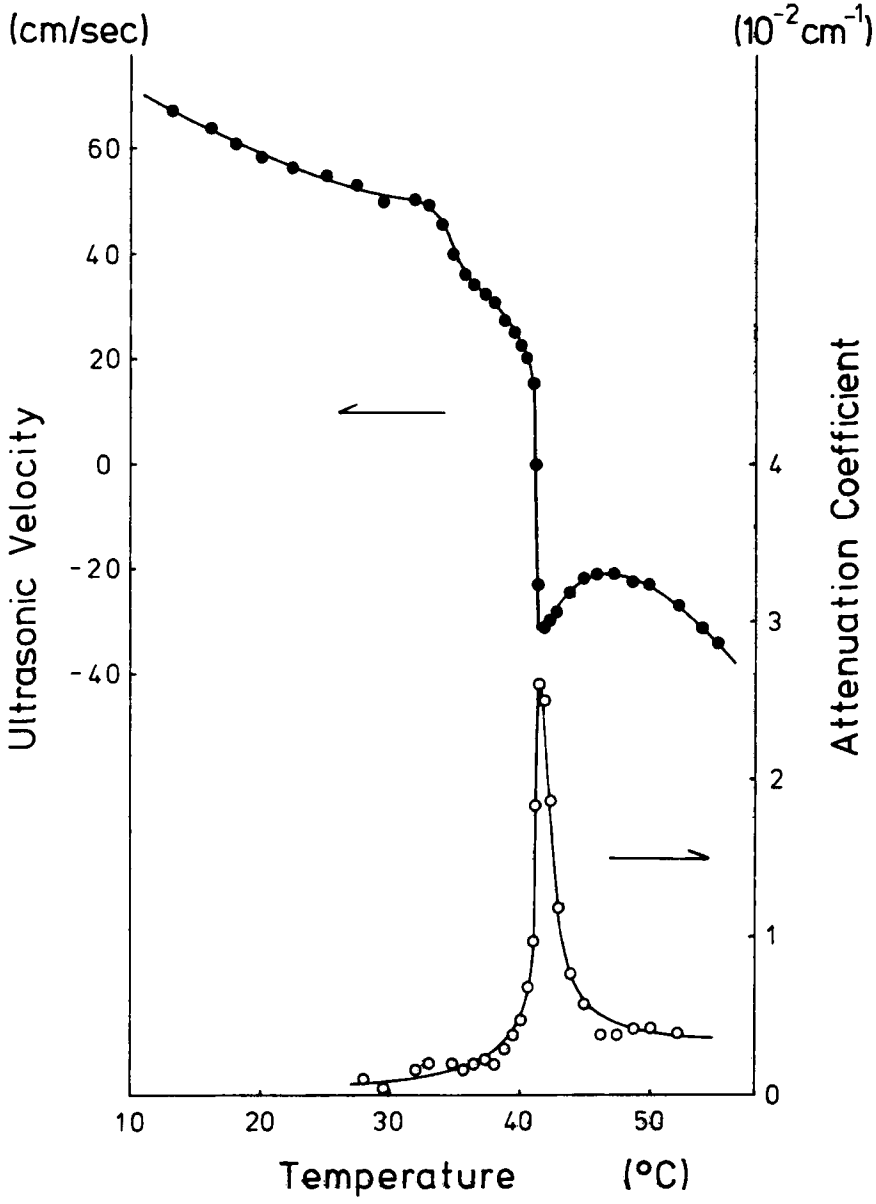

FIGURE 1 The excess ultrasonic velocity and absorption in nonsonicated liposomes of dipalmitoyl phosphatidylcholine are plotted as a function of temperature. The dry weight concentration of the sample is 2.7 mg/ml.

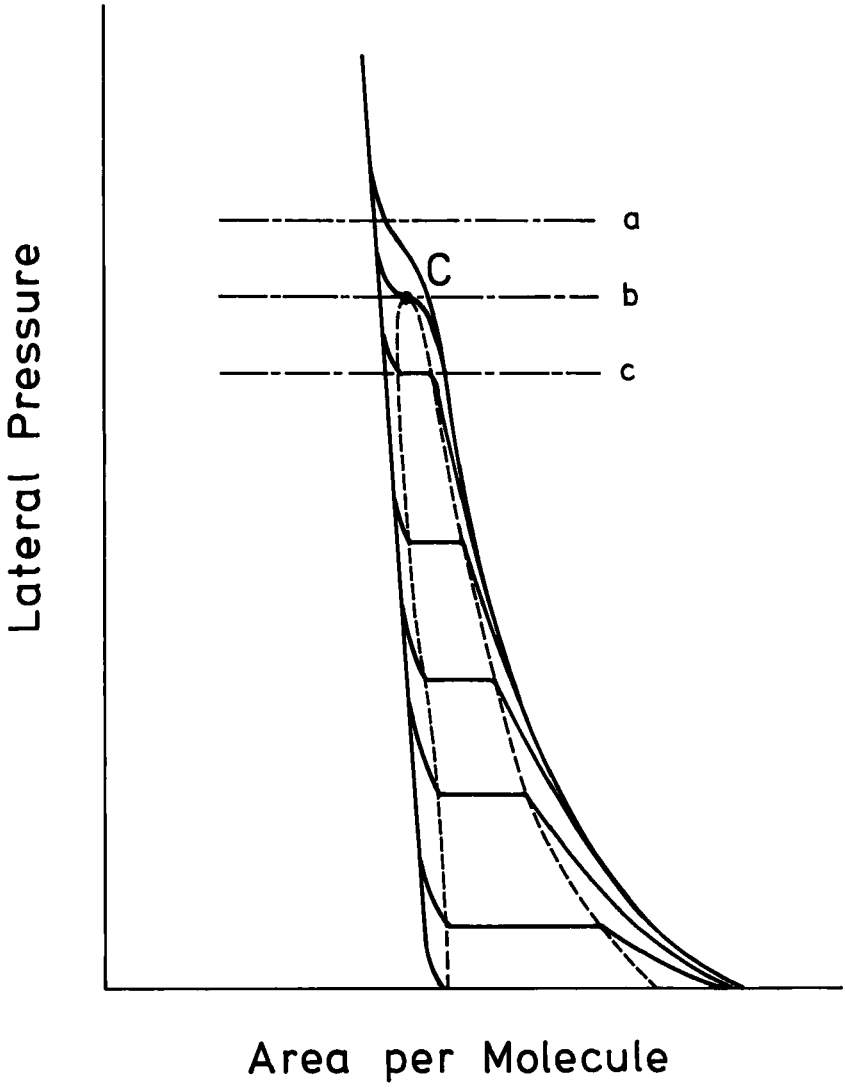

FIGURE 2 Schematic pressure-area phase diagram of dipalmitoyl phosphatidylcholine monolayer, in which *C* denotes a critical point.

the properties of lipid bilayers corresponds to those of lipid monolayers.[8,21] Figure 2 shows schematically the lateral pressure-area phase diagram of dipalmitoyl phosphatidylcholine. Although many minor transitions are reported by Albrecht *et al.*,[8] only main transition of interest is shown in this figure. Solid lines represent isotherms. According to the detailed study by Hui *et al.*[9] and Albrecht *et al.*,[8] there is a critical point where $T_c = 42 \sim 44°C$, $\pi_c =$

47 ~ 50 dyn/cm and $A_c = 40 \sim 50\ A^2$. In the temperature region below T_c, isotherms have two branches of condensed and expanded phases. Dashed lines denote the phase boundaries of two phases and C represents the critical point. In consistent with this pressure-area phase diagram, Jähnig has calculated recently the orientational order parameter of lipid hydrocarbon chains assuming a simple microscopic model and showed that there is a critical point in the lipid bilayer.[22]

There are three different cases for the thermal transition in this system which is represented by broken lines *a*, *b* and *c*. In analogous to van der Waals gases,[23] characteristic ultrasonic behaviors are expected for these three cases. A broad dip of velocity and peak of absorption without any discontinuity or cusp has to be measured in the case of *a*. There will be a deep cusp for *b*. Anomalous dip together with a sharp change of the velocity has to be observed when lipid bilayer undergoes a transition along the line *c*. The present result indicates that the actual dipalmitoyl phosphatidylcholine bilayer corresponds to the case *c*. The transition point of lipid bilayer is probably a little below the critical point of lipid monolayer.[22,24] This conjecture from the transition properties is consistent with the discussion by Nagle[14] and Hui *et al.*[9] Nagle pointed out that the transition point of about 42°C is a little lower than the critical temperature.[21] Hui *et al.* have measured the area per molecule in lipid bilayers which was nearly equal to the critical value of molecular area.[9] Although $\pi\Delta A$ is smaller than the transition enthalpy ΔH as Albrecht *et al.* have pointed out,[8] the contribution of internal energy ΔU to ΔH has to be considerably large: $\Delta H = \Delta(U + \pi A) = \Delta U + \pi\Delta A$. Therefore, small value of $\pi\Delta A$ does not necessarily contradict to the transition near the critical point.

We have measured simultaneously the ultrasonic velocity and absorption for the first time and proposed that the phase transition of nonsonicated dipalmitoyl phosphatidylcholine liposomes is a weak first order transition, and that the transition point of dipalmitoyl phosphatidylcholine bilayer is a little below the critical point of lipid monolayer at the air-water interface. Now we are studying the frequency dispersions of the ultrasonic propagation in the same system as well as in the mixture of various lipids.

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