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Chemistry

Nanosecond relaxation processes of phospholipid bilayers in the transition zone

(thermal transition/chain isomerizations/ultrasonics)

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ABSTRACT **Ultrasonic relaxation spectra of dipalmitoyl lecithin vesicles have been recorded as a function of temperature over the frequency range 14–265 MHz. A relaxation process is observed with a time constant of about 10^{-8} sec. At the midpoint of the crystalline–liquid crystalline transition (about 41.3°), the relaxation amplitude is maximal. This suggests that the relaxation process is intimately associated with the order–disorder transition. Further support for this conclusion comes from the finding that the volume change of the reaction, as calculated from the relaxation amplitude at the transition midpoint, agrees with that determined independently by equilibrium dilatometry measurements of the ΔV of the transition. The results show that a major step in the transition occurs on a far shorter time scale than previously recognized. Similar fast processes have also been detected in dimyristoyl and distearoyl lecithin vesicles. From a consideration of various lines of evidence, it appears that the relaxation monitors the elementary step associated with the isomerization of lipid chains, such as kink formation through internal bond rotations, as the bilayer transforms between ordered and disordered phases.**

The transition of phospholipid bilayers between crystalline and liquid crystalline states has been studied at equilibrium by many methods, including calorimetry (1), dilatometry (2–5), fluorescence probes (5, 6), and magnetic resonance (6–9). In contrast to the many equilibrium investigations, comparatively little has been done to characterize the transient kinetic and mechanistic features of the reaction. Träuble (10) has studied the transition for dipalmitoyl lecithin (DPL) using temperature-jump methods in conjunction with an optical probe. In more recent investigations, turbidity changes have been used to follow the temperature-jump relaxation spectrum (11, 12). In these studies, at least two relaxation processes are observed in the millisecond and longer time range. These data have been interpreted in terms of a nucleation–propagation scheme for the phospholipid bilayer transition (12).

It is plausible to assume that some of the elementary steps associated with the bilayer transition should be considerably faster than the millisecond time range explored by the temperature-jump method. In particular, time constants associated with hydrocarbon chain motion and water structure reorganization may be expected to fall in the range of microseconds to nanoseconds.

It is well known that the changes in hydrocarbon chain packing and water structure give rise to relative volume changes of 2.6–4% when bilayers pass from the crystalline to the liquid crystalline phase (2–5). This substantial volume change means that the transition is ideally suited for study by a pressure-perturbation method. With these considerations in mind, we report here a study of the ultrasonic relaxation spectrum of DPL vesicles in the transition zone. Measurements have been made over the range 15–265 MHz. A process with a relaxation time of about 10 nsec has been characterized. Its amplitude is maximal at the midpoint of the thermal transition, and the volume change estimated from the ultrasonic data agrees closely with that determined independently by dilatometry. Similar relaxation processes in their respective transition zones have also been observed for dimyristoyl and distearoyl lecithin (DML and DSL) vesicles. These results indicate that a key reaction in the structural reorganization of the bilayer occurs on a far shorter time scale than previously recognized (11, 12).

Ultrasonic absorption and dispersion measurements

Stock solutions (10–30 ml) of DPL (α-β dipalmitoyl-L-γ-lecithin; Calbiochem) were made up in 1 mM Na cacodylate (pH 6.5). Vesicles were formed by sonicating these solutions for 2–3 min with a Branson model 1850 cell disrupter, operated at 80 W. A characteristic clearing of the turbid solution occurs when small vesicles are formed (3). Because these vesicles are apparently metastable, stock solutions were always sonicated just prior to use, thus minimizing the formation of lamellar structures during experiments (3).

Ultrasonic relaxation measurements were made by using equipment described by Rhodes and Schimmel (13). When an ultrasonic wave is transmitted through a solution along the x axis, the pressure amplitude P of the wave is attenuated according to the simple relationship (14)

$$P = P_0 e^{-\alpha x} \qquad [1]$$

in which α is the pressure amplitude absorption coefficient and P_0 is the pressure at $x = 0$. For a single relaxation process with a relaxation time τ, the absorption coefficient α varies with frequency f according to

$$\alpha/f^2 = \frac{A\tau}{1 + \omega^2\tau^2} + B \qquad [2]$$

in which A is an amplitude parameter, $\omega = 2\pi f$, and B is the high-frequency limiting absorption (14). An alternative representation of the data makes use of the parameter μ_{chem} which is a measure of the energy absorption per wave length λ due to chemical relaxation processes. The relationship between μ_{chem} and α/f^2 is given by

$$\mu_{chem} = (\alpha/f^2 - B)2vf \qquad [3]$$

in which v is the velocity of sound in solution. When plotted versus frequency, the parameter μ_{chem} shows a peak at the frequency corresponding to the reciprocal relaxation time (14).

The thermal transition of DPL vesicles was monitored by

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Abbreviations: DPL, dipalmitoyl lecithin; DML, dimyristoyl lecithin; DSL, distearoyl lecithin.

turbidity measurements. A midpoint of 41.3° was found, which is close to that determined calorimetrically by Hinz and Sturtevant (1). A similar midpoint was determined from dilatometry measurements. At this temperature an ultrasonic relaxation process with a large amplitude is observed. The amplitude diminishes on either side of the transition temperature of 41.3°.

Fig. 1 is a plot of μ_{chem} versus f for DPL vesicles at 41.3°. Experimental points are given for the frequency range 15–265 MHz. It is clear that the points rise to a maximum at around 15–20 MHz. Error brackets on two of the points illustrate that the error in μ_{chem} increases considerably at high frequencies where the value of α/f^2 approaches B, so that $(\alpha/f^2 - B)$ becomes very small (see Eqs. 2 and 3).

The curve in Fig. 1 is based on a theoretical calculation using Eqs. 2 and 3 with $\tau = 10$ nsec (16 MHz) and $A = 1.2 \times 10^{-7}$ sec cm^{-1}. This curve fits the experimental points reasonably well. It is estimated that the errors in the values of A and τ are $<\pm 15\%$.

A solution containing DPL at $^1/_{10}$ the concentration was also investigated. Absorption measurements were made at a number of frequencies and a 90% reduction in the absorption above background (B) was typically observed. This indicates that the relaxation process in Fig. 1 is due to a unimolecular process, as expected.

Temperature dependence

Ultrasonic relaxation measurements were made on both sides of the thermal midpoint. At temperatures above the midpoint, the amplitude dropped off markedly. On the other hand, the drop-off was not nearly as rapid on the low-temperature side of the transition. This may reflect some asymmetry in the transition.

The parameters obtained are summarized in Table 1. Both the amplitude A/m and relaxation time τ are tabulated (m is the molal concentration of monomer lipid). Parameters obtained at 25° and 45° are subject to considerable error owing to the large decrease in the amplitude. Nevertheless, it is clear from Table 1 that the amplitude sharply diminishes at temperatures on either side of the transition midpoint. On the other hand, the relaxation time is relatively insensitive to temperature. For example, it is identical at 40.0° and 41.3°. In contrast, according to the interpretation of recent temperature-jump data, the millisecond and longer time constants associated with bilayer melting show a significant temperature dependence, particularly for small temperature changes around the transition midpoint (12).

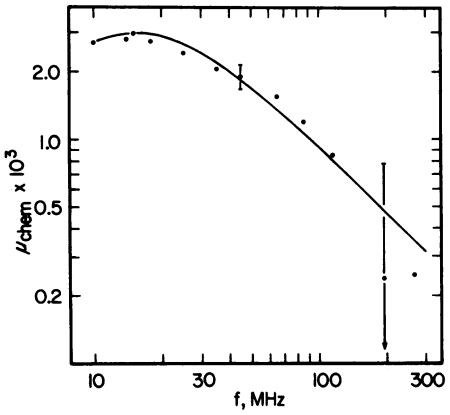

FIG. 1. μ_{chem} versus f for DPL vesicles at a DPL monomer concentration of 7.3 mg/ml in 1 mM Na cacodylate, *ca* pH 6.5, 41.3°. The points are experimental values; the curve is calculated from Eq. 3 with the parameters given in Table 1. Approximate error limits are indicated on two of the experimental points, illustrating how the error in μ_{chem} increases with frequency.

Table 1. Ultrasonic relaxation parameters for DPL vesicles at various temperatures

$10^5 A/m$, sec · m^{-1} · cm^{-1}	τ, nsec	Temperature, °C
(0.48)	(7.2)	25
0.54	15.9	30
1.06	9.9	40
1.21	9.9	41.3
(0.47)	(6.4)	45

All solutions contained DPL monomer at 7.3 mg/ml (or 0.01 molal). Numbers in parentheses are less accurate than those at 40° or 41.3°, owing to the small amplitude.

Volume change

Because the amplitude of the 10-nsec ultrasonic relaxation process peaks at the transition midpoint, it is obvious that this process is intimately associated with the transition. To obtain further insight on the connection between the relaxation process and the transition, the ΔV of the reaction may be calculated from the amplitude parameter A. This is accomplished by utilizing the relationship (14)

$$A = \frac{2\pi^2 \rho v}{RT} \Gamma_m \left[\Delta V - \frac{\alpha_p}{\rho c_p} \Delta H \right]^2 \qquad [4]$$

in which ρ is the solvent density, c_p and α_p are the isobaric specific heat and coefficient of thermal expansion, respectively, and ΔV and ΔH are the volume and enthalpy change of the reaction.

Most of the parameters in Eq. 4 can be determined experimentally or are tabulated elsewhere (15). To calculate ΔV, a value for ΔH must be assumed. We use $\Delta H = 9.7$ kcal (40.5×10^3 kJ)/mol of DPL monomer, determined calorimetrically by Hinz and Sturtevant (1). [However, because the ΔV term dominates, the value of ΔV calculated from the experimental determination of $(\Delta V - \alpha_p/\rho c_p \Delta H)^2$ is fairly insensitive to the exact value chosen for ΔH (14).] The parameter Γ_m has a value of 0.25 m at the midpoint of a unimolecular reaction (14), where m is the molal concentration. Using this value for Γ_m in conjunction with the value for A at 41.3° (see Table 1), we calculate a value of $|\Delta V| = 24.6 \pm 3.0$ cm^3/mol of DPL monomer.

Dilatometry measurements through the transition were made by a method similar to that of Sheetz and Chan (3). From these data, a value of 22.4 ± 3.0 cm^3/mol of DPL monomer was obtained. The close correspondence of the ΔV obtained from the ultrasonic data with that from the equilibrium dilatometry measurements strongly suggests that the relaxation process observed is directly associated with the expansion of the bilayer during the transition.

Ultrasonic relaxation processes in other lecithin bilayers

In order to explore the generality of the effects observed with DPL, studies were also done with bilayers of DML and DSL. By using turbidity measurements, these were determined to have transition midpoints of 24.2° and 54.8°, respectively. These midpoints are close to those determined by Hinz and Sturtevant (1), who worked under similar conditions.

In the case of DPL, the relaxation process is concentration independent and has only a negligible temperature dependence. As a consequence, a plot of $(\alpha/f^2 - B)$ versus tempera-

ture at a fixed frequency (within the range where ultrasonic dispersion occurs) shows a peak at the transition midpoint. If a process analogous to that in DPL vesicles is also present in DML and DSL vesicles, then similar plots of $(\alpha/f^2 - B)$ versus temperature should show peaks at the transition midpoints of these lipids.

Such plots are shown in Fig. 2. $(\alpha/f^2 - B)$ *vs.* temperature is plotted for the fixed frequency of 15 MHz. For all three lipids, $(\alpha/f^2 - B)$ increased sharply to a peak at the transition midpoint and then fell abruptly thereafter. Although their ultrasonic relaxation behavior was not examined in detail, it seems clear that processes analogous to those found for DPL are also present in DML and DSL vesicles.

Two of the curves in Fig. 2 show a small ripple at low temperatures, well below the transition midpoint. This may be due to the presence of additional relaxation processes that are most apparent at temperatures well removed from the transition.

Conclusions

It should be mentioned that Hammes and Roberts (16) investigated the ultrasonic absorption and dispersion of phosphatidylserine and lecithin suspensions in approximately the same frequency range as studied here. Concentration-independent relaxation effects were observed but were not studied as a function of temperature or in the transition zone.

There seems to be little question that the relaxation process at about 10 nsec in DPL vesicles is associated with the order–disorder transition. This conclusion is based on two pieces of evidence. First, the amplitude of the relaxation process peaks at the midpoint of the transition, as is expected if the relaxation monitors the transition reaction (14). Second, the ΔV of the relaxation effect corresponds closely with the ΔV associated with the transition.

Temperature-jump measurements have shown the existence of at least two relaxation processes in the range of milliseconds and longer (11, 12). The relaxation times seem to show a significant temperature dependence. In particular, both relaxation times appear to peak at the transition temperature. This behavior has been interpreted as consistent with a lattice dynamic model of bilayers and the transition (12).

The temperature-jump experiments utilized turbidity measurements. Apparently, most of the turbidity changes that occur as a result of the transition are accounted for by relaxations that occur in times of $>10^{-3}$ sec (12). These turbidity changes are presumably associated with the cooperative rearrangement of large clusters of lipid molecules.

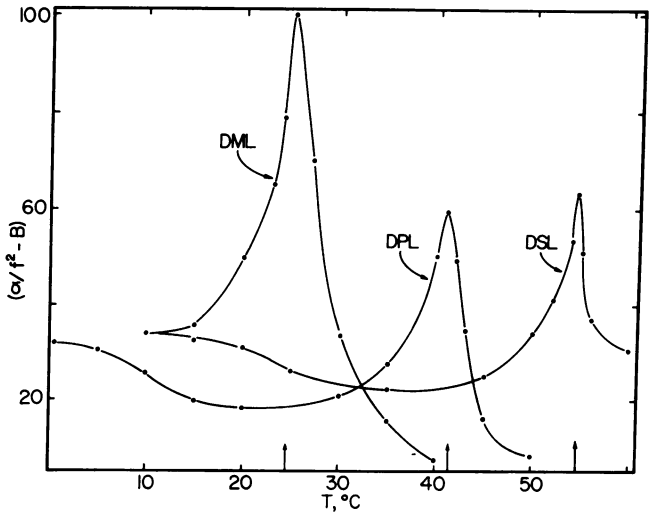

FIG. 2. $(\alpha/f^2 - B)$ at 15 MHz versus temperature for vesicles of DML (8.0 mg/ml), DPL (7.3 mg/ml), and DSL (6.7 mg/ml), each in 1 mM Na cacodylate buffer, *ca* pH 6.5. Arrows along the abscissa indicate the transition midpoint temperatures which were determined independently by turbidity measurements.

The close agreement between the ΔV (per monomer) of the 10-nsec relaxation studied here and the ΔV (per monomer) of the transition suggests that we are observing the elementary step associated with the isomerization of lipid chains, such as kink formation through internal bond rotations, as the bilayer transforms between ordered and disordered phases. Certainly, the tumbling times and lateral diffusion rates of electron spin resonance labeled lipids are in the approximate time range of the ultrasonic relaxation process studied here (6, 17). But, more to the point, internal rotations in aliphatic chains that give rise to disordered forms are expected to occur in this time range (13, 18, 19). In addition, rotational barrier heights for polymethylene-type chains are low (20), which is consistent with our observations of little temperature variation in the ultrasonic relaxation time. Thus, it is plausible that chain isomerizations are responsible for the ultrasonic relaxation effect. These isomerizations may be closely tied to the kinking reactions discussed by Petersen and Chan (21).

Chain isomerizations presumably go on at temperatures well below the transition zone, albeit less frequently. This would explain our ability to observe some relaxation amplitude at 25°, well below the 41.3° transition midpoint. As the temperature is increased, changes in the interactions within large clusters of lipid chains inside the bilayer alter the equilibrium between "crystalline" and disordered forms so as to give a more equitable distribution of these isomers; accordingly, there is an increase in the relaxation amplitude to the point at which it peaks at the transition midpoint. Above the midpoint, the disordered forms predominate and the amplitude diminishes.

To summarize, the present results can be readily explained as arising from elementary chain isomerization reactions of individual lipid hydrocarbons. Some reorganization of water structure may simultaneously occur and contribute to the ΔV of the reaction. On the other hand, the concerted reorganization of large clusters of molecules within the bilayer occurs on a much longer time scale and gives rise to turbidity changes.

It should be mentioned that the high-frequency limiting absorption at *ca* 265 MHz is close to, but not quite equal to, the background absorption of water. This implies that no additional relaxation processes of substantial amplitude occur at higher frequencies. Thus, unless there is a reaction whose equilibrium cannot be perturbed by an ultrasonic wave, there are no additional relaxation processes of significant amplitude beyond 150 MHz ($\tau \approx 1$ nsec).

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