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Stopped-flow rapid kinetics of anesthetic-induced phase transition in phospholipid vesicle membranes: nonlocalized fluctuation

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Kinetics of the gel to liquid-crystalline phase transition of dipalmitoylphosphatidylcholine vesicle membrane was studied by the stopped-flow technique with turbidity detection. The observed change in turbidity was well characterized by a single-exponential decay curve with relaxation time in the millisecond range, although the existence of a faster process than the dead-time of the stopped-flow apparatus was inferred from the amplitude analysis. Relaxation times were determined as functions of 1-hexanol concentration and temperature just below phase transition. From the analysis based on the theories of nonequilibrium relaxation, it is concluded that the phase transition induced by 1-hexanol is governed by a nonlocalized fluctuation mechanism. The anesthetic-induced nonequilibrium state is unstable rather than metastable.

Introduction

Phospholipid membranes undergo a first-order gel-to-liquid-crystalline phase transition, depending on external parameters such as temperature, pressure, etc. Phase transition is also affected by the presence of small ligand molecules, such as anesthetics. A number of reports demonstrated that anesthetics depressed the phase-transition temperature (see, for instance, a review by Ueda and Kamaya [1]). Depression of the transition temperature, induced by anesthetics, is often considered to be directly or indirectly related to anesthesia mechanisms, although specific receptor concepts have also been proposed. Reports on anesthetic actions on lipid phase-transition, however, are limited to the equilibrium properties. The kinetic aspects have received little attention. It is expected that kinetic studies may provide information concerning the mechanism of anesthetic-induced phase transition.

Kinetic studies on phase transition of phospholipid vesicle membranes have been reported using chemical relaxation techniques, such as temperature jump [2–5], pressure jump [6,7], and ultrasonic absorption [8–11]. The only exception appears to be the study reported by Strehlow and Jähnig [12], in which phase transition was induced by changing the pH of a suspension of phosphatidic acid vesicles; i.e., the phase transition was triggered electrostatically through the change in the degree of ionization of the lipid head group.

When a system with a first-order transition is transferred into a nonequilibrium state by a sudden change in an external parameter, the relaxation of the system into a new equilibrium state can

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be classified into two different types, according to whether the nonequilibrium state is unstable or metastable. The first is caused by nonlocalized (long wavelength) fluctuation and the second by localized fluctuation (nucleation) [13,14]. Strehlow and Jähnig [12] analyzed the relaxation time associated with the pH-jump-triggered phase transition in the phosphatidic acid system, and reported that the relaxation process in this system is governed by localized fluctuations.

Because anesthetic molecules depress the transition temperature, the phase transition from the gel state to the liquid-crystalline state can be triggered by mixing the lipid suspension with anesthetic solution at an isothermal condition. The equilibrium effect of 1-alkanols on the phase transition of dipalmitoylphosphatidylcholine (DPPC) vesicle membranes has been studied by several authors [15–19]. In the present communication, we report the kinetics of the phase transition of DPPC vesicle membranes induced by 1-hexanol, measured by a stopped-flow technique. The vesicle suspension was rapidly mixed with 1-hexanol solution, and the time-course of the phase transition was monitored by a turbidity change. By the mixing, the system in a gel-rich equilibrium is suddenly brought into an environment where the liquid-crystalline-rich state is stable. In this process, the transition from the nonequilibrium gel state to the equilibrium liquid-crystalline state is observed.

Experimental

Materials. Synthetic dipalmitoylphosphatidylcholine (DPPC) and 1-hexanol were obtained from Sigma. Water was triply distilled, once from alkaline potassium permanganate solution.

DPPC vesicle suspension in water was prepared by sonication in the cuphorn of a Branson Sonifier Model 185 (Danbury, CT) at above the phase transition temperature. After sonication, vesicle suspension was aged at 4°C for two weeks to fuse into relatively homogeneous size according to the report by Wong et al. [20].

Methods. A Durrum Model D-110 stopped-flow spectrophotometer was used for the kinetic measurements. DPPC vesicle suspension in water was mixed with 1-hexanol aqueous solution with a mixing ratio of 1 : 1. The initial DPPC concentration before mixing was 0.74 mM. The temperature of the sample, controlled by circulating water from a constant temperature water bath around the flow system, was maintained within ± 0.05 deg. C of the desired temperature.

The phase-transition process of the vesicle after mixing was followed by the absorbance change at 360 nm. The output signal of the spectrophotometer was stored in a Nicolet Model 3091 digital oscilloscope and recorded on a Hewlett-Packard XY recorder. The detecting system was calibrated so that the output signal of 10.0 V from the photomultiplier through the log amplifier corresponds to 1.00 absorbance. The absorbance shown in Figs. 1–3 was obtained by converting the output signal in volts on the oscilloscope display to the absorbance unit.

The experiment was repeated at least four runs and the data were stored in an Apple IIe microcomputer interfaced with a PDP 11/23. Data reduction, error computation, and linear and polynomial curve fittings were performed with a statistics program.

Results and Discussion

The present stopped-flow experiment consisted of varying a 1-hexanol concentration under the fixed DPPC concentration at several temperatures.

Fig. 1a illustrates a typical time-course of the absorbance change after mixing DPPC with a 1-hexanol solution. The lower curve in Fig. 1a was obtained by mixing the DPPC suspension with pure water. At the initial stage after mixing, the instability in absorbance was observed. From the instability, the dead-time of the stopped-flow apparatus was estimated to be about 4 ms. The decrease in absorbance with time (the upper curve in Fig. 1a) indicates that the lipid in the initial equilibrium gel-state transforms into a liquid-crystalline form and relaxes to a new equilibrium state.

The decay was well described by a single exponential curve as shown by a semilogarithmic plot in Fig. 1b, where the rapid signal change within the dead-time was omitted.

Two informations are obtainable from the present experiment. One is the final equilibrium state expressed by the absorbance at $t = \infty$ (A_∞ in Fig.

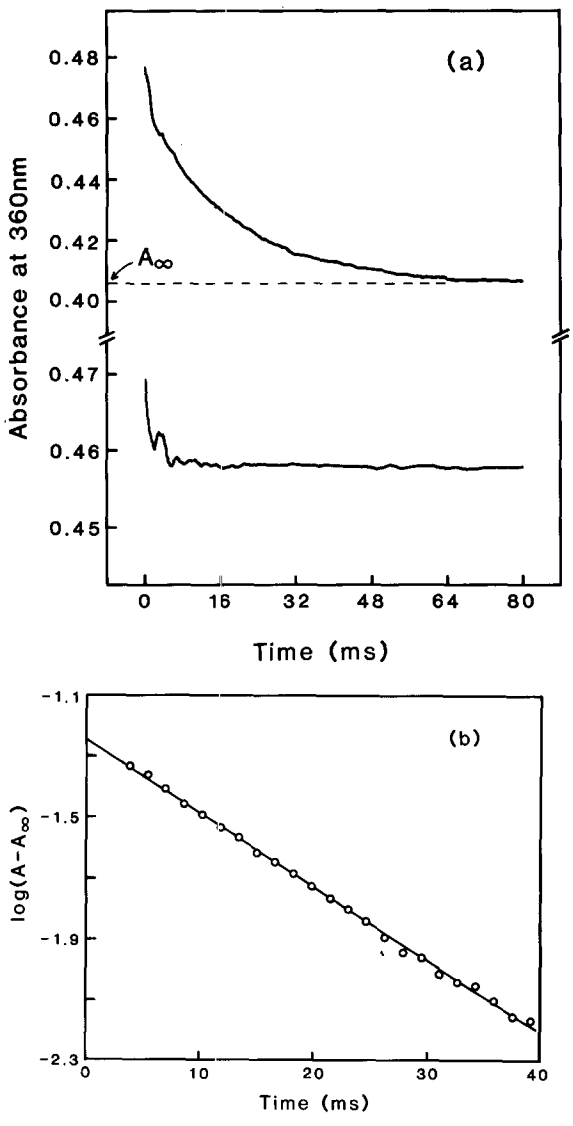

Fig. 1. (a) A typical time-course of absorbance change after mixing 0.74 mM DPPC suspension with 6.32 mM 1-hexanol solution (upper curve) and with water (lower curve) at 40.0°C. (b) Semilogarithmic plot of the upper curve in (a), where A and A_∞ represent the absorbance at time t and infinity, respectively.

1a) (static information); the other is the relaxation time characterizing the rate of the phase transition (kinetic information).

Fig. 2 shows a temperature dependence of the absorbance of pure DPPC suspension, which was determined by mixing the DPPC suspension with water (the lower curve in Fig. 1a) at various temperatures. In Fig. 2, high absorbance at temperatures below 39°C corresponds to the gel state and low absorbance above 43°C corresponds to the liquid-crystalline state. The phase transition temperature was estimated to be 41.3°C, which is in good agreement with the literature [21].

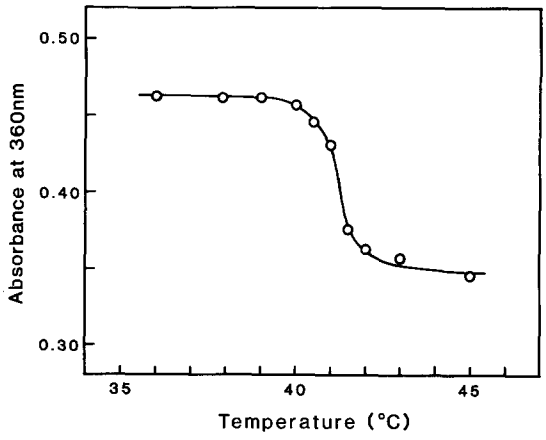

Fig. 2. Plot of absorbance at 360 nm of 0.37 mM DPPC suspension against temperature. Absorbance was determined by mixing 0.74 mM DPPC suspension with water in a stopped-flow apparatus (see lower curve in Fig. 1a).

In Fig. 3, the absorbance at the final equilibrium state, obtained from the mixing experiments, is plotted against 1-hexanol concentration, C_A, at several temperatures around the phase transition. It is clearly shown in Fig. 3 that the phase transition from the gel to the liquid-crystalline state is induced by 1-hexanol at constant temperature. It is also seen that the phase-transition concentration of 1-hexaol, which corresponds to the midpoint of the transition curve, is shifted to lower concentration with increasing temperature.

The relaxation curve, such as the one shown in Fig. 1a, was obtained at 1-hexanol concentrations that induce phase transition. The relaxation process was characterized by a single-relaxation time constant, τ, which was obtained from the slope of semilogarithmic plot (Fig. 1b). Fig. 4 shows the dependence of τ on temperature and 1-hexanol concentration. The relaxation time constant decreased monotonously with the increase in 1-hexanol concentration.

Fig. 5 shows the results of amplitude analysis. In this figure, ΔA_s represents the statically determined amplitude, i.e., the difference in absorbance between the initial state ($t = 0$) and the

final state ($t = \infty$); the former is the absorbance at $C_A = 0$, and the latter corresponds to the absorbance at the final equilibrium. The kinetically determined amplitude is expressed by ΔA_k, and was estimated by extrapolating the straight line of the semilogarithmic plot (Fig. 1b) to $t = 0$. The ratio, $\Delta A_k/\Delta A_s$, was unity when the 1-hexanol concentration was below the phase-transition value. With increasing 1-hexanol concentration, the ratio started to decrease when the 1-hexanol concentration exceeded the phase-transition value. The value of $\Delta A_k/\Delta A_s$ less than 1.0 suggests the existence of a fast process above the phase transition concentration. The process is too fast to be resolved by a stopped-flow technique. This is contrary to the case of pH-jump-induced phase transition of the phosphatidic acid membranes where monophasic kinetics was demonstrated [12].

In a stopped-flow study, it was reported [22] that 1-hexanol was not incorporated into the membrane core but bound to near the membrane surface. Then the energy barrier for the 1-hexanol binding is expected to be small, so that the binding may be close to a diffusion-controlled process. Based on this consideration, it may be reasonably assumed that the binding equilibrium of 1-hexanol to the lipid membrane is attained rapidly. Hence, the observed relaxation process can be regarded as

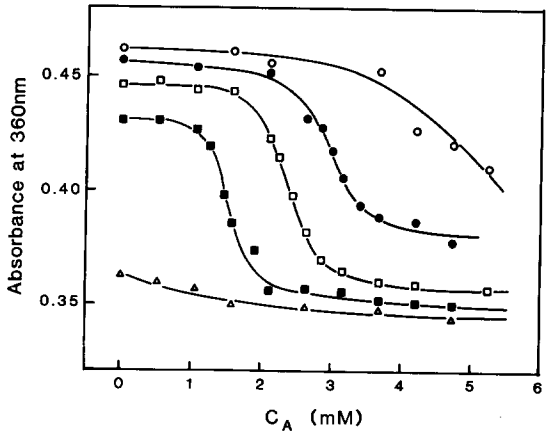

Fig. 3. Plot of absorbance at 360 nm against 1-hexanol concentration at different temperatures. Each point shows the absorbance at final equilibrium obtained from kinetic measurement (A_∞ in Fig. 1a). C_A represents 1-hexanol concentration after mixing. Temperature (°C) is 39.0 (○), 40.0 (●), 40.5 (□), 41.0 (■), and 42.0 (△).

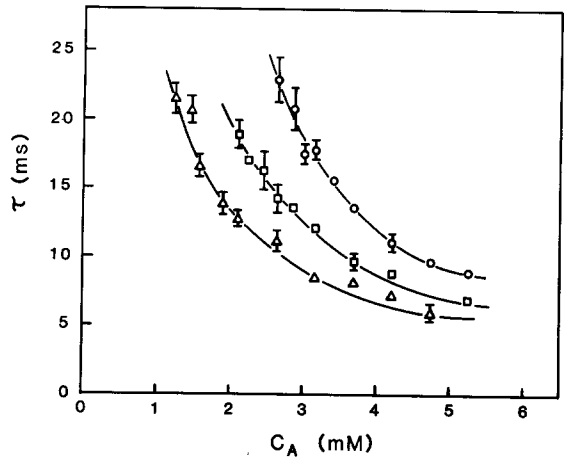

Fig. 4. Plot of relaxation time, τ, against 1-hexanol concentration, C_A, at different temperatures. Temperature (°C) is 40.0 (○), 40.5 (□), and 41.0 (△). Vertical bars indicate the standard deviation ($n = 5$).

a phenomenon directly associated with the gel-to-liquid phase transition of the lipid membrane.

We now analyze the relaxation time, τ, of the gel-liquid phase transition, triggered by a sudden change in an external parameter, x, along the lines of the theoretical model of Strehlow and Jähnig [12]. According to their treatment, τ is related to the displacement of the nonequilibrium state from the phase transition point, which is characterized by the difference of the external parameter from that at the transition point.

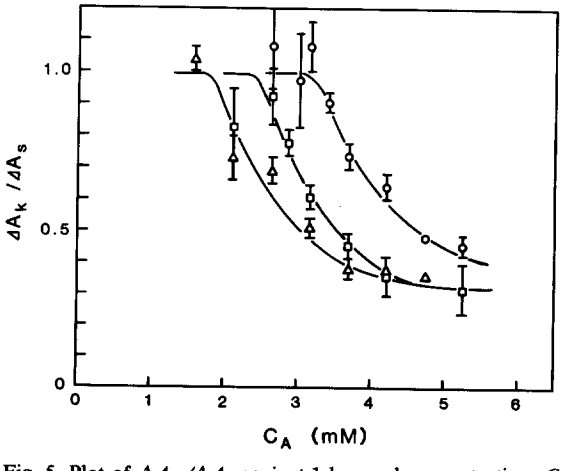

Fig. 5. Plot of $\Delta A_k/\Delta A_s$ against 1-hexanol concentration, C_A, at different temperatures. Signs are the same as Fig. 4. Vertical bars represent the standard deviation ($n = 5$). See text for the meaning of $\Delta A_k/\Delta A_s$.

If the relaxation process is governed by non-localized fluctuations, τ is related to the displacement by

$$\tau \propto |x - x^t|^{-1/2} \tag{1}$$

whereas, if the relaxation is caused by localized fluctuations, the relation becomes

$$\ln \tau \propto |x - x^t|^{-1} \tag{2}$$

where superscript t stands for the value of x at the phase transition point. Eqn. 1 can be derived from the time-dependent Ginzburg-Landau theory [13], whereas Eqn. 2 is derived from the classical nucleation theory [12]. Strehlow and Jähnig [12] applied the above relations to the pH-triggered phase transition by identifying x with the electrostatic surface pressure, π_{el}, defined by $\pi_{el} = -\partial F_{el}/\partial a$, where F_{el} is the electrostatic free energy caused by the charges of the lipid head group, and a is the area of the membrane.

In the present system, in which the lipid membrane is in contact with a 1-hexanol solution of concentration C_A, the free energy F of the system is given by the sum

$$F = F_0 + F_{bin} \tag{3}$$

where F_0 is the free energy in the absence of 1-hexanol and F_{bin} is the free-energy increase due to the binding of 1-hexanol molecules to the membrane. If we assume that the binding is of the Langmuir type, then F_{bin} can be expressed as

$$F_{bin} = -N_m kT \ln(1 + KC_A) \tag{4}$$

where N_m is the number of binding sites of the membrane, k is the Boltzmann constant, T is the absolute temperature, and K is the binding constant of 1-hexanol to the membrane. For low C_A (in accordance with the present experimental condition), Eqn. 4 may be approximated by

$$F_{bin} = -N_m kTKC_A \tag{5}$$

O'Leary [23] has proposed two models of the anesthetic adsorption to the lipid membrane, in which N_m or K is assumed to be a linear function of a, the area of the membrane consisting of a given number of lipid molecules. This assumption is formulated as

$$N_m = N_0 + \alpha(a - a_0) \tag{6}$$

or

$$K = K_0 + \beta(a - a_0) \tag{7}$$

where α and β are proportionality constants, and N_0 and K_0 corresponds to N_m and K, respectively, when a is equal to a reference value, a_0. From Eqns. 5 and 6, or from Eqns. 5 and 7, one finds that the surface pressure arising from the 1-hexanol binding, π_{bin}, is proportional to C_A; i.e.,

$$\pi_{bin} = -(\partial F_{bin}/\partial a) \propto C_A \tag{8}$$

Noting that π_{bin} (and hence C_A) corresponds to the external parameter x in the present system, we obtain from Eqns. 1 and 2

$$\tau \propto |C_A - C_A^t|^{-1/2} \tag{9}$$

for the nonlocalized fluctuation mechanism, and

$$\ln \tau \propto |C_A - C_A^t|^{-1} \tag{10}$$

for the localized fluctuation mechanism, where C_A^t represents the transition-inducing concentration of 1-hexanol defined as the midpoint of the plot between the equilibrium absorbance value and the 1-hexanol concentration (Fig. 3).

In order to examine which of the two fluctuation mechanisms governs the 1-hexanol-triggered phase transition of the DPPC membrane, the experimental values of τ were plotted according to Eqn. 9 (in Fig. 6a) and Eqn. 10 (in Fig. 6b). In Fig. 6, only the data points corresponding to the plateau region in Fig. 3 were plotted, where the phase transition to the liquid-crystalline state is complete, because within the transition region, the phenomenon becomes complicated by the reverse reaction (i.e., liquid-crystalline to gel). The values of C_A^t were determined from the transition curve in Fig. 3, as follows; C_A^t = 2.98 mM (40.0°C), 2.36 mM (40.5°C), and 1.52 mM (41.0°C).

These figures show that τ is proportional to $(C_A - C_A^t)^{-1/2}$ (the data points in Fig. 6a lie on a straight line through the origin) but $\ln \tau$ is not proportional, indicating that the relaxation mechanism in the present system is governed by non-

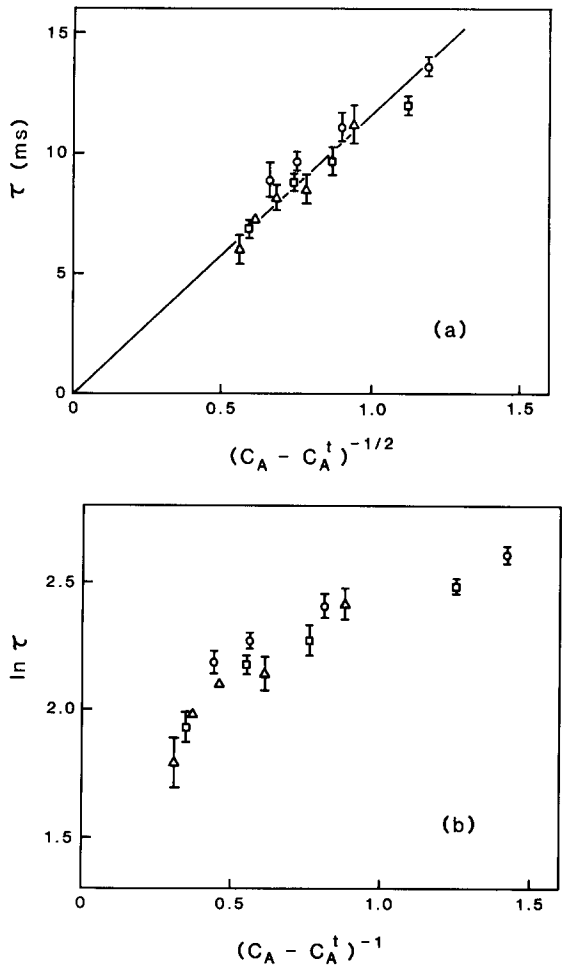

Fig. 6. Relation between the relaxation time, τ, and $C_A - C_A^t$. The relaxation time is plotted according to Eqn. 9 (a) and Eqn. 10 (b). C_A^t is the concentration of 1-hexanol corresponding to the midpoint of the transition curve plotted between the 1-hexanol concentration and the equilibrium absorbance value. Signs are the same as Fig. 4. Vertical bars represent the standard deviation ($n = 5$).

localized fluctuations. This result contrasts with the observation of Strehlow and Jähnig [12] on the pH-triggered lipid phase transition, where the transition followed the localized fluctuation mechanism. This discrepancy, however, is not a contradiction. Instead, the present result, together with the result of Strehlow and Jähnig [12], suggest that the relaxation process is not inherent to the lipid membrane itself but depends strongly on the external parameter triggering the phase transition.

It has been shown theoretically [13,14] that relaxation from the nonequilibrium state to the equilibrium state with a nonlocalized fluctuation mechanism is observed if the nonequilibrium state is unstable, whereas with a localized fluctuation mechanism is observed if the nonequilibrium state is metastable. The present results indicate that the nonequilibrium state induced by 1-hexanol, which is attained rapidly within a dead-time of the stopped-flow apparatus, is unstable.

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