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Effects of pressure and local anesthetic tetracaine on dipalmitoylphosphatidylcholine bilayers

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Abstract

The temperature-pressure phase diagram of dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles was constructed in the presence of a local anesthetic tetracaine hydrochloride (TC-HCl). The phase-transition temperatures under various pressures were determined by the method of high-pressure light transmission. The temperature of the main transition from the ripple gel (P'_β) to the liquid crystal (L_α) phase was depressed by the addition of TC-HCl and elevated by application of pressure up to 150 MPa. The temperature of the pretransition from the lamellar gel (L'_β) to the P'_β phase was also depressed by the addition of TC-HCl below ca. 10.0 mmol kg^{-1} and elevated by the pressure below ca. 50 MPa. Therefore, pressure-anesthetic antagonism for both phase-transitions was confirmed. The pressure-induced interdigitated gel ($L_\beta I$) phase has been observed under high pressure above 100 MPa in the absence of TC-HCl. The $L_\beta I$ phase is known to be induced also by a variety of small amphiphilic molecules such as ethanol, benzyl alcohol and TC-HCl. In the presence of TC-HCl ranging in concentration up to 20.0 mmol kg^{-1}, the $L_\beta I$ phase instead of the P'_β phase appeared at higher pressure. Present results revealed that pressure facilitates, rather than antagonizes, the effect of TC-HCl on the occurrence of interdigitated gel phase. Furthermore, two regions of two phase coexistence were observed under high pressure in the presence of TC-HCl. One is probably a region of coexisting $L_\beta I$ and L_α phase, which was found between $L_\beta I$ and L_α phases under various pressures. The other is probably a region of coexisting L'_β and $L_\beta I$ phase, which was observed in the presence of TC-HCl up to 10.0 mmol kg^{-1} at the pressure above 40 MPa and at the temperature below ca. 35°C.

Keywords: Lipid bilayer; Vesicle; Phase transition; Interdigitation; Local anesthetic; Pressure reversal

1. Introduction

As regards the thermodynamic understanding of any biochemical systems, the study of the effect of temperature under ambient pressure has been most common although the pressure is also an important thermodynamic variable. High pressure studies on gene, protein and lipid membrane systems have been initiated in the last decade and provided much useful physicochemical understanding of these systems [1]. Each of these vital constituents exhibits a different receptivity to hydrostatic pressure. Although DNA molecules are stable under high pressure up to 1000 MPa, some proteins are denatured by pressure above 300 MPa and lipid bilayer membrane undergoes barotropic phase transition from the liquid-crystalline

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to solid-gel states below 100 MPa. Therefore, lipid bilayer membranes constructing the biomembrane responds most sensitively to hydrostatic pressure.

Anesthetics are known as a typical drug which acts directly on the biomembranes. The mechanism of anesthetic action is still uncertain, but the site of this action is generally presumed to be the cellular membrane of the neuron and the anesthetic potency is well known to be correlated with the anesthetic partitioning into membranes. The pressure reversal of anesthesia both in vivo [2–4] and in vitro [5–13] has been physiologically interesting. The investigation of the pressure-anesthetic antagonism has been regarded as a possible key to elucidate the mechanism of anesthetic action. Anesthetics increase the volume of the lipid bilayer membranes [6] and its fluidity [7], while pressure is presumed to reverse both of these effects.

It is well known that dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles undergo two phase transitions with increasing temperature at ambient pressure; one is the main transition from the ripple gel (P'_β) phase to the liquid crystalline (L_α) phase and the other is the pretransition from the lamellar gel (L'_β) phase to the P'_β phase. Hydrostatic pressure compresses the lipid bilayer anisotropically; lipid bilayers are laterally compressible while the acyl chains straighten and slightly extend the thickness of the bilayer. Consequently, the phase-transition temperatures of DPPC vesicles were elevated by the pressure and depressed by the addition of anesthetics [8–13]. A number of studies have been made on the phase behavior of DPPC multilamellar vesicles and recent studies have suggested that there exist a new interdigitated gel ($L_\beta I$) phase induced by high pressure in addition to three phases mentioned above [14–18].

Auger and co-workers have reported the pressure-induced exclusion of a local anesthetic tetracaine from lipid bilayers by the method of a high-pressure Fourier transform infrared spectroscopy [19]. They have also revealed that the uncharged tetracaine disorders the lipid acyl chains of dimyristoylphosphatidylcholine bilayers in the gel state while the charged form of tetracaine induces the formation of the $L_\beta I$ phase [20]. Driscoll et al. have shown by high-pressure ^{2}H-NMR techniques that the order parameter of all segments of the acyl chains increases with pressure in the liquid crystal state of DPPC bilayer membrane. The addition of tetracaine increases the disorder of the chains, and the pressure reverses the effect of anesthetic on the lipid [21]. Peng and Jonas [22] studied the structure and dynamics of the phosphocholine head group in DPPC bilayers in the absence and presence of tetracaine by using high-pressure ^{31}P-NMR techniques. They showed that the addition of tetracaine increased the ^{31}P chemical shift anisotropy and brought about a change in the conformation of the head group which swung toward the bilayer normal, from its usual orientation parallel to the membrane surface. The addition of tetracaine also induced the formation of the $L_\beta I$ phase directly from the L_α phase. However, there is little information about the influence of local anesthetics on the phase behavior of lipid bilayer membranes although it seems to be useful for a better understanding of the pressure-anesthetic antagonism.

In the present study, to elucidate the effects of pressure and anesthetics on a model biomembrane system, we examined the phase behavior of DPPC vesicles in the absence and presence of local anesthetic tetracaine hydrochloride (TC-HCl) under high pressure. The phase transition was observed by the method of high-pressure light transmission [18].

2. Materials and methods

Tetracaine-HCl (TC-HCl), 2-dimethylaminoethyl-4-butylaminobenzoate hydrochloride, was obtained from Sigma Chemicals (St. Louis, MO) in the crystalline form and recrystallized several times from ethanol. For the study of the anesthetic effect, TC-HCl was first dissolved in distilled water.

DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, was purchased from Sigma Chemicals (St. Louis, MO) and used directly without further purification. According to the suppliers, the purity is better than 99%. Lipid powder was first suspended in TC-HCl solution to give a lipid concentration of 2.0 mmol kg^{-1}. Measurements were made on TC-HCl solutions ranging in concentration up to 20.0 mmol kg^{-1}. The chemical structures of the local anesthetic and the phospholipid used are shown in Fig. 1. This anesthetic-lipid suspension was sonicated by using a Branson Sonifier Model 185 and a cup horn at temperatures several degrees above the main-transition

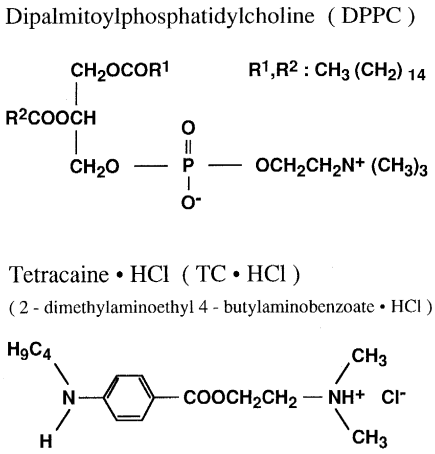

Fig. 1. Molecular structures of phospholipid and local anesthetic tetracaine.

temperature for 5 min, in order to prepare the phospholipid multilamellar vesicles [23,24]. The sample was incubated at 45°C for at least 10 h before measurement in high-pressure vessel. The phase transition temperatures were determined by observing the transmittance at a certain pressure during the course of heating with scanning rate of 0.67 K min^{-1}, after a glass syringe cell filled the sample solution was placed in the optical high-pressure vessel. The transmittance as a function of temperature was followed at 540 nm. The general arrangement of the high pressure apparatus for the phase transition measurements has been described previously [25].

The phase transitions of DPPC bilayer membrane in the presence of TC-HCl under ambient pressure were observed by a Micro Cal MCS high-sensitivity differential scanning calorimeter (Northampton, MA, USA). The heating and cooling rates were usually 0.75 K min^{-1} and occasionally 0.5 or 0.25 K min^{-1}.

3. Results and discussion

Differential scanning calorimetry (DSC) was used for the first time to investigate the effect of TC-HCl on the phase-transition temperatures for DPPC bilayer membranes under ambient pressure. Fig. 2 shows typical DSC thermograms of DPPC bilayer membranes at various TC-HCl concentrations. Two kinds of transitions were observed at ambient pressure in curves a and b. Higher-temperature transition can be assigned to the main transition from the P'_β phase to the L_α phase. On the other hand, lower-temperature transition is assigned to the pretransition from the L'_β phase to the P'_β phase. The peak of main transition was gradually broadened as TC-HCl concentration increased. In the presence of 23.0 mmol kg^{-1} TC-HCl (curve c), lower-temperature transition could be assigned to the transition from the $L_\beta I$ phase to the P'_β phase as discussed below. Curve d shows both heating and cooling scans of the main transition from $L_\beta I$ to L_α and vice versa. As can be seen, this transition has a hysteresis between heating and cooling scans. The scanning rate in the range of 0.25 to 0.75 K min^{-1} did not affect the present results. This hysteresis was observed remarkably in the presence of TC-HCl more than 23.0 mmol kg^{-1}.

Fig. 3 shows the phase transition temperatures of DPPC bilayer membranes as a function of TC-HCl concentration up to 60.0 mmol kg^{-1}. The main-transition temperature (T_m) and pretransition temperature (T_p) in the absence of TC-HCl were observed at 41.2°C and 34.1°C, respectively, which were in good agreement with previously reported data [26,27]. Both T_m and T_p were gradually decreased with an increase in TC-HCl concentration, and TC-HCl decreased effectively T_p rather than T_m. It is noteworthy that the $L_\beta I$ phase instead of the P'_β phase appears at the

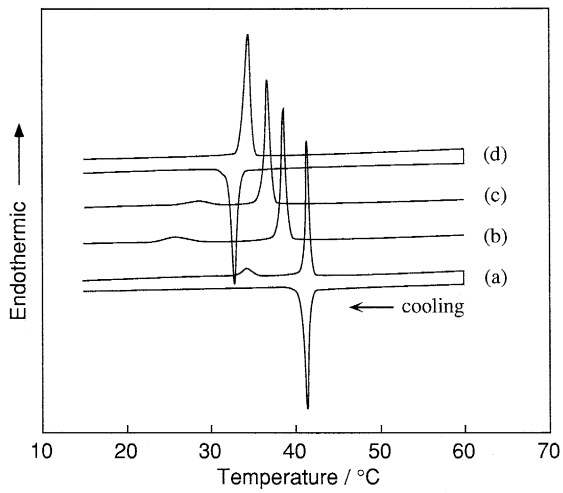

Fig. 2. DSC heating and cooling curves of DPPC vesicle suspensions at various concentrations of TC-HCl: (a) without TC-HCl, (b) 10.0 mmol kg^{-1} TC-HCl, (c) 23.1 mmol kg^{-1} TC-HCl and (d) 49.8 mmol kg^{-1} TC-HCl. (a) and (d) show both scans of heating and cooling. DPPC concentration is 2.0 mmol kg^{-1}.

concentration of TC-HCl above ca. 21 mmol kg^{-1}. Interdigitation of DPPC bilayer has been observed by the addition of ethanol [28–30], 1-butanol [31] and other surface-active small molecules [32–34].

A phase boundary between L'_β and $L_\beta I$ phases was observed at a narrow range of TC-HCl concentration around 21 mmol kg^{-1}. This concentration can be regarded as the critical concentration for the interdigitation of DPPC bilayer. With respect to the phase boundary between L_α and $L_\beta I$ phases, there was the temperature gap between heating and cooling scans. This hysteresis may be attributable to a different receptivity to tetracaine partitioning between L_α and $L_\beta I$ phases. As more tetracaine molecules are dissolved into the L_α phase of DPPC bilayer, the temperature of transition on cooling from L_α to $L_\beta I$ phase would be lower than that on heating from $L_\beta I$ to L_α phase. The depression of the phase-transition temperature by additives can be regarded as a thermodynamic colligative property. Therefore, we could observe the larger depression of the phase-transition temperature on the cooling scan in comparison with that on the heating scan, because of higher concentration of tetracaine in the L_α phase than the $L_\beta I$ phase.

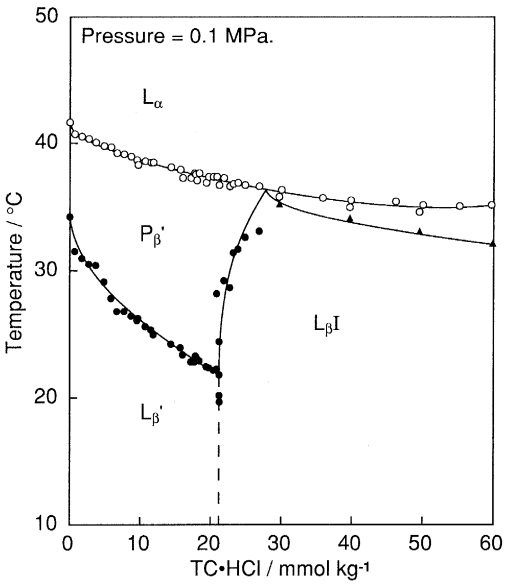

Fig. 3. Phase transition temperatures of DPPC bilayer membranes determined by the DSC method as a function of TC-HCl concentration. DPPC concentration is 2.0 mmol kg^{-1}. Phase transition: (○) $P'_\beta \rightarrow L_\alpha$ or $L_\beta I \rightarrow L_\alpha$, (●) $L'_\beta \rightarrow P'_\beta$ or $L_\beta I \rightarrow P'_\beta$, (▲) $L_\alpha \rightarrow L_\beta I$ on cooling scans.

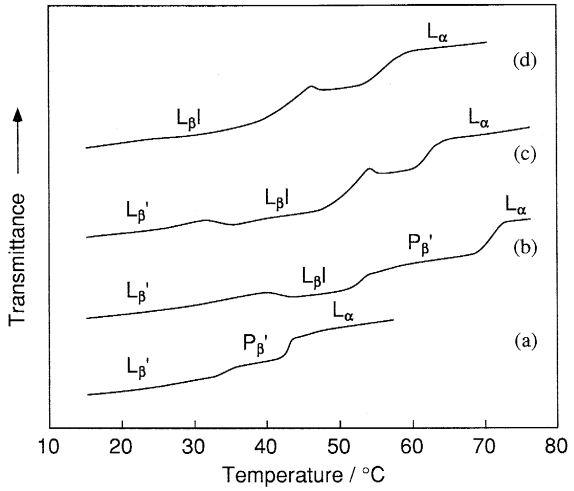

Fig. 4. Some typical transmittance-temperature curves of DPPC vesicles at (a) 0.1 MPa without TC-HCl, (b) 125 MPa without TC-HCl, (c) 100 MPa with 10.0 mmol kg^{-1} TC-HCl and (d) 75 MPa with 20.0 mmol kg^{-1} TC-HCl.

Some typical transmittance-temperature curves of DPPC vesicle suspensions in the absence and the presence of TC-HCl are shown in Fig. 4. Two kinds of transitions were observed at ambient pressure in the absence of TC-HCl (curve a), which can be assigned to the main transition at 41.2°C and the pretransition at 34.1°C. Both transition temperatures were in good agreement with DSC data. Under high pressure (curve b), the L'_β phase was transformed to the pressure-induced $L_\beta I$ phase around 40°C. The transition from the L'_β phase to the $L_\beta I$ phase is accompanied with an increase in turbidity, which may be caused by increased molecular packing in multilamellar vesicles. This observation agrees with the report that the specific volume of DPPC in the $L_\beta I$ phase is less than that in the L'_β phase [30,35]. In the presence of 10.0 and 20.0 mmol kg^{-1} TC-HCl, the transmittance-temperature curves under high pressure (curves c and d in Fig. 4) showed a new step via a local maximum in transmittance with increasing temperature. It seems as if a new transition induced by the local anesthetic was observed distinctly [36].

The temperature-pressure phase diagram of DPPC multilamellar vesicles in the absence of local anesthetic is shown in Fig. 5. The temperatures of the main transition and the pretransition were increased linearly by pressure. It was observed that the $L_\beta I$ phase appears at a pressure beyond 100 MPa [14,16].

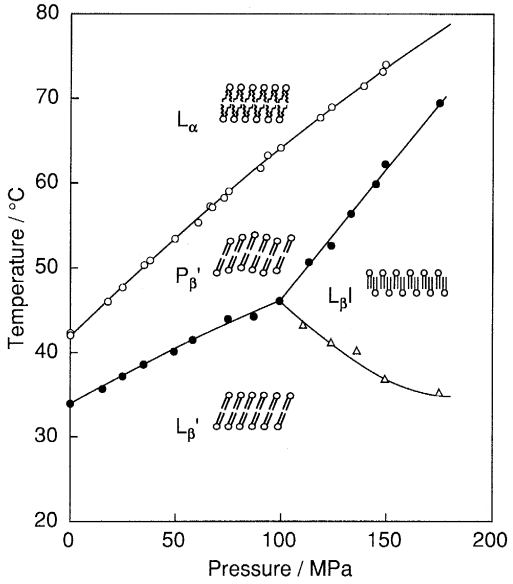

Fig. 5. Temperature-pressure phase diagram of DPPC bilayers at 2.0 mmol kg^{-1}. Phase transition: (●) $L'_\beta \rightarrow P'_\beta$ or $L_\beta I \rightarrow P'_\beta$, (△) $L'_\beta \rightarrow L_\beta I$, (○) $P'_\beta \rightarrow L_\alpha$.

The lipid bilayer structures in the L_α, P'_β, L'_β and $L_\beta I$ phases are also illustrated in Fig. 5.

Figs. 6 and 7 show the phase diagrams of DPPC vesicles in the presence of 5.0 and 10.0 mmol kg^{-1} TC-HCl, respectively. T_m and T_p were depressed by the addition of TC-HCl at constant pressure and elevated almost linearly by pressure. It was confirmed that the $L_\beta I$ phase appeared at high pressure above 70 MPa in the presence of 5.0 mmol kg^{-1} TC-HCl and above 45 MPa in the presence of 10.0 mmol kg^{-1} TC-HCl. Consequently, the appearance of the $L_\beta I$ phase was extended to lower pressure ranges by the addition of local anesthetic in a dose-dependent manner, in comparison with the DPPC phase diagram in the absence of TC-HCl. The decrease in partial molar volume of DPPC molecule due to interdigitation is a response to compression. DPPC bilayers go into the $L_\beta I$ phase by compression, and simultaneously cause an increase in density of lipid bilayer membrane and in van der Waals contacts of acyl chain region. On the other hand, amphiphilic molecules such as tetracaine anchor to the interface by virtue of their polar moiety, with the non-polar part of the molecule intercalating between the gel state hydrocarbon chains. These results clearly indicate that the formation of the $L_\beta I$ phase is promoted mutually by the addition of local anesthetic and applying pressure, that is, pressure facilitates rather than antagonizes the effect of TC-HCl on the

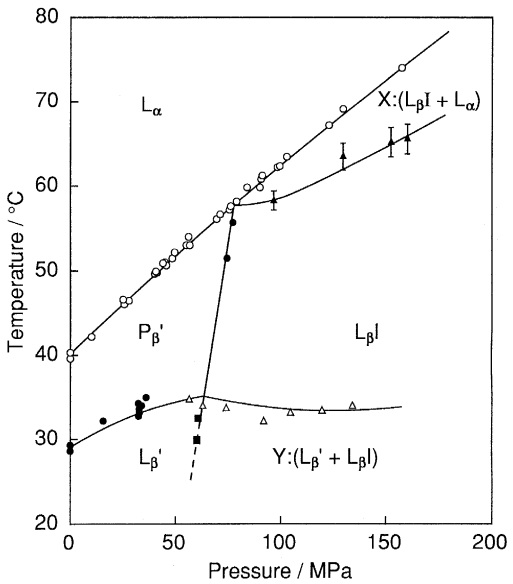

Fig. 6. Temperature-pressure phase diagram of DPPC bilayers in the presence of 5.0 mmol kg^{-1} TC-HCl. Phase transition: (■) $Y \rightarrow L'_\beta$, (●) $L'_\beta \rightarrow P'_\beta$ or $L_\beta I \rightarrow P'_\beta$, (△) $Y \rightarrow L_\beta I$, (▲) $L_\beta I \rightarrow X$, (○) $P'_\beta \rightarrow L_\alpha$ or $X \rightarrow L_\alpha$.

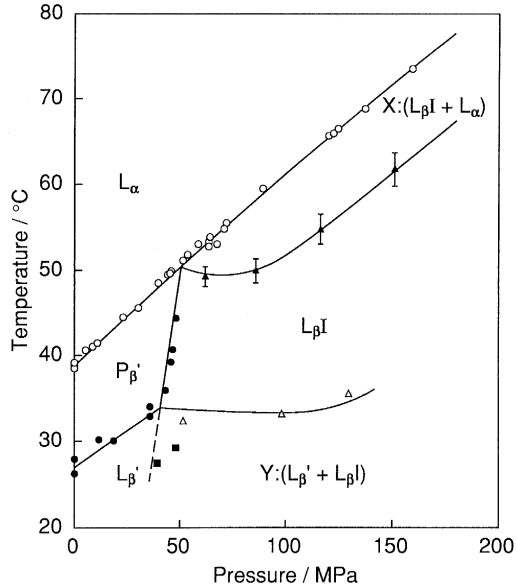

Fig. 7. Temperature-pressure phase diagram of DPPC bilayers in the presence of 10.0 mmol kg^{-1} TC-HCl. Phase transition: (■) $Y \rightarrow L'_\beta$, (●) $L'_\beta \rightarrow P'_\beta$ or $L_\beta I \rightarrow P'_\beta$, (△) $Y \rightarrow L_\beta I$, (▲) $L_\beta I \rightarrow X$, (○) $P'_\beta \rightarrow L_\alpha$ or $X \rightarrow L_\alpha$.

occurrence of the $L_\beta I$ phase. Therefore, it is presumed that the region of the $L_\beta I$ phase may be extended to lower pressure ranges by more addition of local anesthetic tetracaine.

As is seen from curve c in Fig. 4, a new transition via a local maximum in transmittance was observed under high pressure above ca. 50 MPa in the presence of 5.0 to 10.0 mmol kg^{-1} TC-HCl. As shown in the phase diagrams of Figs. 6 and 7, a new region X exists between $L_\beta I$ and L_α phases instead of the P'_β phase. The X region on the phase diagram is considered as follows. Taking into account the application of the phase rule to the present system, it is difficult to identify a new region X on the phase diagram as a single new phase. If the X region on the phase diagram is assigned as a single new phase, it is unlikely that it is a point of four-phase coexistence on the phase diagram. Since the X region can be observed distinctly between $L_\beta I$ and L_α phases under high pressure and the temperature of transition between $L_\beta I$ and L_α phases exhibits a thermal hysteresis between heating and cooling scans, the region of X on the phase diagram can be identified as a $L_\beta I$-L_α two-phase coexistent region. Because the transformation of bilayers from the $L_\beta I$ phase to the L_α phase is accompanied by the compositional change of anesthetic in bilayer membranes, rearrangement of bilayers seems to be responsible for a local maximum in the transmittance vs. temperature profile shown in Fig. 4. In addition, another new region Y on the phase diagram shown in Figs. 6 and 7 can be identified as the L'_β-$L_\beta I$ two-phase coexistent region, which will be mentioned below by the temperature-composition diagrams under high pressures.

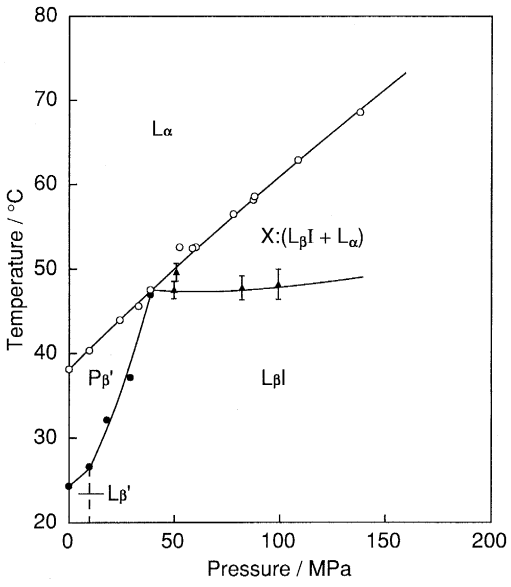

Fig. 8. Temperature-pressure phase diagram of DPPC bilayers in the presence of 15.0 mmol kg^{-1} TC-HCl. Phase transition: (●) $L'_\beta \rightarrow P'_\beta$ or $L_\beta I \rightarrow P'_\beta$, (▲) $L_\beta I \rightarrow X$, (○) $P'_\beta \rightarrow L_\alpha$ or $X \rightarrow L_\alpha$.

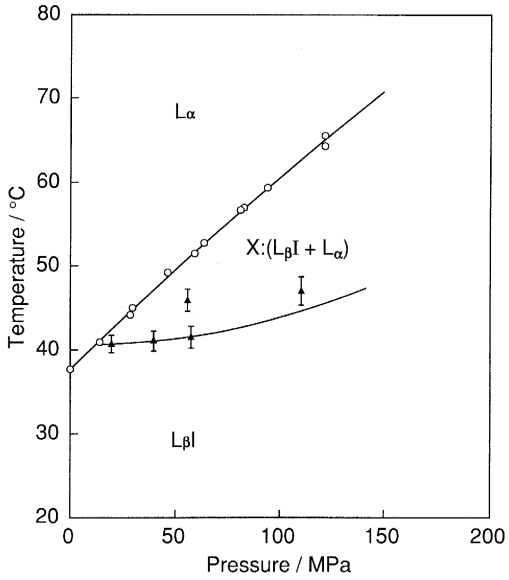

Fig. 9. Temperature-pressure phase diagram of DPPC bilayers in the presence of 20.0 mmol kg^{-1} TC-HCl. Phase transition: (▲) $L_\beta I \rightarrow X$, (○) $L_\beta I \rightarrow L_\alpha$ or $X \rightarrow L_\alpha$.

Figs. 8 and 9 show the phase diagrams of DPPC vesicles in the presence of 15.0 and 20.0 mmol kg^{-1} TC-HCl, respectively. T_m was depressed by the addition of TC-HCl at constant pressure and elevated gradually by applying pressure at constant concentration of local anesthetic. It was confirmed that the X region of two-phase coexisting appeared at the pressure above 50 MPa and 20 MPa in the presence of 15.0 and 20.0 mmol kg^{-1} TC-HCl, respectively. Consequently, the existent region of X was extended to lower pressure regions by more addition of local anesthetic, in comparison with the phase diagram (Fig. 7) in the presence of 10.0 mmol kg^{-1} TC-HCl. Moreover, as is seen from Fig. 9, DPPC bilayers existed in the $L_\beta I$ phase at ambient pressure above ca. 20.0 mmol kg^{-1} TC-HCl and underwent the main

transition from the $L_\beta I$ phase to the L_α phase. Other phase transition phenomena, except for the main transition from the $L_\beta I$ phase to the L_α phase, were not observed at ambient pressure in the TC-HCl concentration above 20.0 mmol kg^{-1}.

As was seen previously from Fig. 3, the results of high sensitivity DSC show that the pretransition from the L'_β phase to the P'_β phase can be observed till the tetracaine concentration of 21.0 mmol kg^{-1}. Endothermic peak of pretransition on DSC thermograms tends to shrink with increasing concentration of the tetracaine and ultimately disappears. Because of the different sensitivity between light transmittance and DSC methods, the faint pretransition in the presence

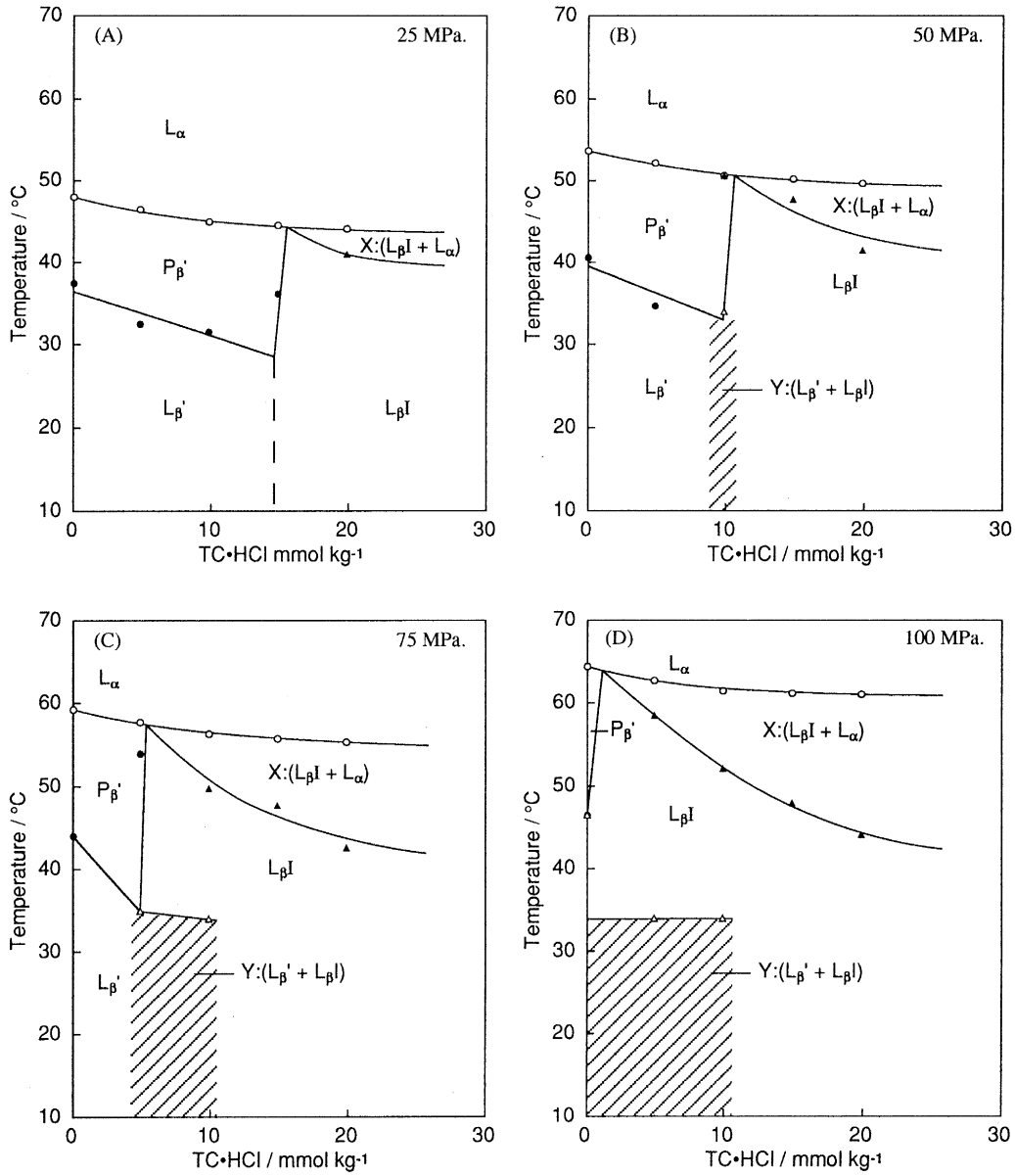

Fig. 10. Temperature-concentration diagrams of DPPC bilayer as a function of TC · HCl concentration at various pressures: (A) 25 MPa, (B) 50 MPa, (C) 75 MPa and (D) 100 MPa. Phase transition: (●) $L'_\beta \rightarrow P'_\beta$ or $L_\beta I \rightarrow P'_\beta$, (△) $Y \rightarrow L_\beta I$, (▲) $L_\beta I \rightarrow X$, (○) $P'_\beta \rightarrow L_\alpha$ or $X \rightarrow L_\alpha$.

of 20.0 mmol kg^{-1} TC-HCl was not detected by an optical method. Taking into consideration the difference in sensitivity between the high-pressure experiment and DSC method, it may be concluded that the results obtained from the high-pressure experiments shown in Figs. 8 and 9 correspond to the results of DSC with regard to the appearance of the $L_\beta I$ phase.

Fig. 10 shows the temperature-concentration diagrams at various constant pressures, which are constructed through the phase diagrams shown in Figs. 5–9. Boundary lines between various phases in Fig. 10 were decided by reference to the phase diagram at ambient pressure shown in Fig. 3. It is clearly shown that the temperatures of the main transition from the P'_β phase to the L_α phase are depressed by the addition of tetracaine and elevated by pressure. The regions of P'_β and L'_β phases on the diagram decrease with an increase in pressure. Contrary to behavior of P'_β and L'_β phases, the region of $L_\beta I$ phase is extended to lower concentration of TC-HCl as pressure increases. In other words, the critical concentration of TC-HCl for the interdigitation of DPPC bilayer decreases with an increase in pressure. Therefore, we may say that tetracaine stabilizes the interdigitated phase of DPPC bilayer. The $L_\beta I$ phase of DPPC bilayer was induced by pressure above 100 MPa or by the addition of TC-HCl more than 21 mmol kg^{-1}. Synergistic effect of tetracaine and pressure on the interdigitation of DPPC bilayer was observed for the transition between L'_β and $L_\beta I$ phases. At the temperature below 34°C, the coexisting region Y of two phases, which is shown by hatched regions on the diagram, was observed at pressure above 50 MPa and was extended to lower concentration of TC-HCl as pressure increased. It will be noted that the concentration more than 10 mmol kg^{-1} TC-HCl is required for the perfect transformation from the L'_β phase to the $L_\beta I$ phase.

In conclusion, as regards the phase transitions of DPPC bilayers, the main-transition and pretransition temperatures were depressed by the addition of local anesthetic tetracaine and elevated by pressure, therefore, the pressure-anesthetic antagonism were observed for these transitions. On the other hand, since the interdigitated gel phase was induced by both pressure and tetracaine, the $L_\beta I$ phase would not be concerned in the pressure-anesthetic antagonism. Moreover, coexisting regions of two phases, i.e., L_α-$L_\beta I$ and L'_β-$L_\beta I$ phases, were confirmed with the aid of phase rule by a mapping of phase boundaries in temperature-pressure-concentration diagrams.

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