ELSEVIER

Biophysical Chemistry 87 (2000) 25–36

Biophysical Chemistry

www.elsevier.nl/locate/bpc

Effect of local anesthetics on the bilayer membrane of dipalmitoylphosphatidylcholine: interdigitation of lipid bilayer and vesicle–micelle transition

Takashi Hata, Hitoshi Matsuki, Shoji Kaneshina*

Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, Minamijosanjima, Tokushima 770-8506, Japan

Received 17 January 2000; received in revised form 29 May 2000; accepted 7 June 2000

Abstract

The phase transitions of dipalmitoylphosphatidylcholine (DPPC) bilayer membrane were observed by means of differential scanning calorimetry (DSC) as a function of the concentration of local anesthetics, dibucaine (DC·HCl), tetracaine (TC·HCl), lidocaine (LC·HCl) and procaine hydrochlorides (PC·HCl). LC·HCl and PC·HCl depressed monotonously the temperatures of the main- and pre-transition of DPPC bilayer membrane. The enthalpy changes of both transitions decreased slightly with an increase in anesthetic concentration up to 160 mmol kg^{-1}. In contrast, the addition of TC·HCl or DC·HCl, having the ability to form a micelle by itself, induced the complex phase behavior of DPPC bilayer membrane including the vesicle-to-micelle transition. The depression of both temperatures of the main- and pre-transition, which is accompanied with a decrease in enthalpy, was observed by the addition of TC·HCl up to 21 mmol kg^{-1} or DC·HCl up to 11 mmol kg^{-1}. The pretransition disappeared when these concentrations of anesthetic were added, and the interdigitated gel phase appeared above these concentrations. The appearance of the interdigitated gel phase, instead of the ripple gel phase, brings about the stabilization of the gel phase by 1.8–2.4 kcal mol^{-1}. In the concentration range of 70–120 mmol kg^{-1} TC·HCl (or 40–60 mmol kg^{-1} DC·HCl), the enthalpy of the main transition exhibited a drastic decrease, resulting in the virtual disappearance of the main transition. This process includes the decrease in vesicle size with increasing anesthetic concentration, resulting in the mixed micelle of DPPC and anesthetics. Therefore, in this range of anesthetic concentration, the DPPC vesicle solubilized an anesthetic which coexists with the DPPC–anesthetic mixed micelle. Above the concentration of 120 mmol kg^{-1} TC·HCl (or 60 mmol kg^{-1} DC·HCl), there exists the DPPC–anesthetic mixed micelle. Two types of new transitions concerned with the mixed micelle of DPPC and micelle-forming anesthetics were observed by DSC.

Keywords: Bilayer membrane; Interdigitation; Lipid bilayer; Local anesthetics; Phase transitions; Vesicle–micelle transition

* Corresponding author. Tel.: +81-88-656-7513; fax: +81-88-655-3162.
E-mail address: kanesina@bio.tokushima-u.ac.jp (S. Kaneshina).

0301-4622/00/$ - see front matter
PII: S0301-4622(00)00175-7

1. Introduction

Anesthetics are known as a typical drug which acts directly on the biomembranes [1]. The molecular mechanism of anesthetic action is still uncertain, but the site of this action is generally presumed to be the cellular membrane of the neuron. In order to elucidate the molecular interactions between biological membranes and anesthetic molecules, many physico-chemical approaches have been reported using model biomembranes such as phospholipid bilayers. In general, the effect of drugs on the biological membrane has been discussed on fluidity or phase transition of the membrane. A few studies on the phase behavior of the phospholipid bilayer in the presence of anesthetics have been reported by physico-chemical methods. A representative phospholipid, dipalmitoylphosphatidylcholine (DPPC) has been widely used in the study of the model biomembranes. It is well known that the DPPC bilayer membrane undergoes three phase transitions with increasing temperature; the subtransition from the lamellar crystal (L_c) phase to the lamellar gel (L_β') phase, the pretransition from the L_β' phase to the ripple gel (P_β') phase and the main transition from the P_β' phase to the liquid crystal (L_α) phase [2–5]. The phase transition temperatures of DPPC bilayer membrane were depressed by the addition of anesthetics in a dose-dependent manner [6–14]. The difference in this temperature-depression dependent on anesthetics has a good relation with the anesthetic potency, therefore, this phenomenon may suggest an important relation with the anesthetic mechanism. On the other hand, the phase transition temperatures were elevated by the pressure, and the pressure-anesthetic antagonism has been reproduced by using the model biomembranes [15–20]. Recent studies on the phase behavior of DPPC bilayer membrane have suggested that there exists an interdigitated gel (L_βI) phase induced by high concentration of alcohols [14,21–24] and local anesthetics [25–27] as well as high pressure [28–33]. However, there is little information about the influence of local anesthetics on the phase behavior of DPPC bilayer membranes [27], although it seems to be useful for better understanding of anesthetic mechanisms.

In the present study, the effect of local anesthetics, i.e. dibucaine (DC·HCl), tetracaine (TC·HCl), lidocaine (LC·HCl) and procaine hydrochlorides (PC·HCl), on the phase behavior of DPPC bilayer membrane has been investigated by the differential scanning calorimetry (DSC). Since some local anesthetics are known to form micelle in the aqueous solution [34–37], the vesicle–micelle equilibrium in the presence of local anesthetics around the critical micelle concentration (CMC) is also elucidated by means of the light scattering technique in view of the colloidal aspects.

2. Materials and methods

2.1. Materials

Four local anesthetic hydrochlorides,

- dibucaine: 2-butoxy-*N*-(2-diethylaminoethyl)-4-quinolinecarboxamide (DC·HCl);
- tetracaine: 2-(dimethylamino)ethyl-4-(butylamino)benzoate (TC·HCl);
- lidocaine: 2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide (LC·HCl); and
- procaine: 2-(diethylamino)ethyl-*p*-(amino)benzoate (PC·HCl)

were obtained from Sigma Chemicals (St. Louis, MO, USA) in the crystalline form and recrystallized several times from ethanol. For the study of the anesthetic effect, these local anesthetics were first dissolved in distilled water.

Synthetic DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine was purchased from Sigma Chemicals and used without further purification. According to the suppliers, the purity is better than 99%. Lipid powder was first suspended in various anesthetic solutions to give a lipid concentration of 2.0 mmol kg^{-1}. Measurements were made on these anesthetic solutions ranging in concentration up to 90.0 (for DC·HCl) or 160.0 mmol kg^{-1} (for other anesthetics). Chemical

structures of phospholipid and local anesthetics used are shown in Fig. 1. This anesthetic–lipid suspension was sonicated weakly by using a Branson Sonifier Model 185 and a cup horn at a temperature several degrees above the main-transition temperature for 5 min, in order to prepare the phospholipid multilamellar vesicles suitable for the phase transition measurements [32,33]. Before the measurements, the suspension was degassed for 3 min.

2.2. Differential scanning calorimetry

The phase transitions of DPPC bilayer membrane in the presence of local anesthetics were observed by a MicroCal MCS high-sensitivity differential scanning calorimeter (Northampton, MA, USA). The heating and cooling rates were 0.75 K min^{-1}.

2.3. Electrical conductivity measurement

The electrical conductivity of DPPC multilamellar vesicle suspension, in the presence of local anesthetics, was measured with a Denki Kagaku Keiki AOL-40 conductivity meter (DKK Corporation, Japan). The cell constant was calibrated with a standard potassium chloride solu-

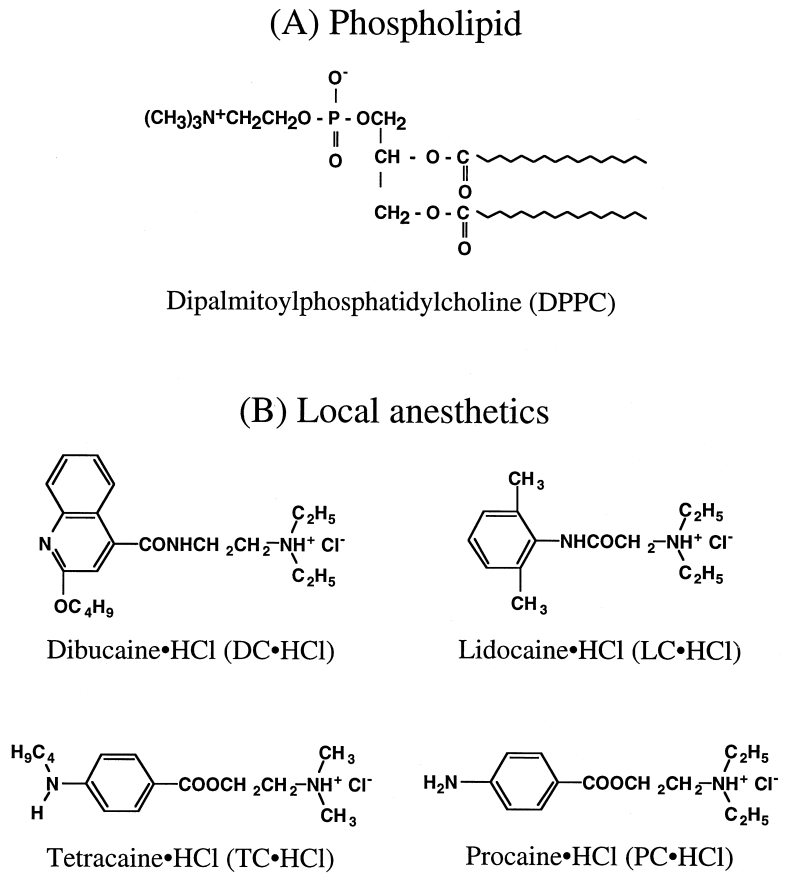

Fig. 1. Molecular structures of (A) phospholipid and (B) local anesthetics.

tion. Temperature of conductivity cells was raised at the same rate of DSC heating scan, 0.75 K min^{-1}, by circulating water from a thermocontroller YSC-9012 (Yamashita Giken, Tokushima, Japan).

2.4. Dynamic light scattering

The average size of DPPC–TC·HCl aggregates was measured by a dynamic light-scattering spectrophotometer DLS-7000 (Otsuka Electronics, Osaka, Japan) with a He–Ne laser operating at 633 nm. All measurements were made at temperatures of 35 and 45°C, maintained by a recirculating water bath. The intensity autocorrelation function was analyzed by the method of histograms. Stock solutions were filtered through 0.45 μm Millipore filters prior to each measurement.

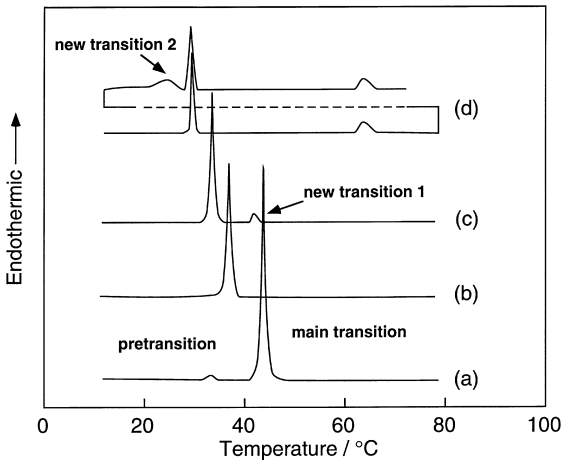

Fig. 2. DSC heating thermograms of DPPC bilayer membrane at several TC·HCl concentrations: (a) without TC·HCl, (b) 20.0 mmol kg^{-1} TC·HCl, (c) 83.0 mmol kg^{-1} TC·HCl and (d) 120.0 mmol kg^{-1} TC·HCl. Thermograms (d) include the reheating scan. DPPC concentration is 2.0 mmol kg^{-1}.

3. Results and discussion

3.1. Effect of TC·HCl on DPPC bilayer membrane

Fig. 2 shows typical DSC thermograms of DPPC bilayer membranes at several concentrations of TC·HCl. The temperatures of main transition (T_m) and pretransition (T_p) in the absence of TC·HCl were observed at 41.2 and 34.1°C, respectively, which are in good agreement with previous data [2–5]. The endothermic peak of the main transition was observed in all the thermograms and became broader as the TC·HCl concentration increases. T_m was depressed by the addition of TC·HCl. T_p was also depressed. The endothermic peak of the pretransition became smaller by the addition of TC·HCl and has disappeared at the concentration above 21 mmol kg^{-1}. In the presence of higher concentration of TC·HCl, we found two types of new transitions. One is the transition observed at a higher concentration than 71 mmol kg^{-1} TC·HCl, which is called the new transition 1. The temperature of new transition 1 was elevated steeply as the concentration of TC·HCl increases. The other is that the transition appeared on the second heating scan at a concentration higher than 110 mmol kg^{-1} TC·HCl, which is called the new transition 2.

Expanded DSC thermograms of DPPC bilayer membrane are shown in Fig. 3 in the range of temperature below the main transition. In a narrow range of the TC·HCl concentration from 21 to 27 mmol kg^{-1}, two types of small endothermic peaks on the DSC thermogram were observed. Since the L_βI phase is known to be induced by the addition of tetracaine as well as ethanol and several surface active small molecules [25], this observation may be attributed to the appearance of the L_βI phase instead of the L_β' phase and the disappearance of the P_β' phase. Ohki et al. [22] have observed the analogous phase transition of DPPC bilayer membrane in the presence of ethanol.

The phase transition temperatures of DPPC bilayer membranes are shown in Fig. 4 as a function of TC·HCl concentration up to 160 mmol kg^{-1}. Both T_m and T_p were gradually depressed by an increase in TC·HCl concentration, and TC·HCl depressed effectively T_p rather than T_m. Because the pretransition depends on the surface structure of the membrane, the anesthetics seem to be affected strongly on the surface structure of

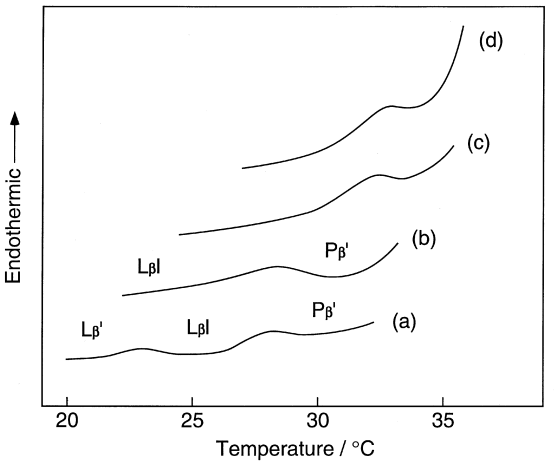

Fig. 3. Appearance of an interdigitated gel phase on DSC thermograms of DPPC bilayer membrane at various TC·HCl concentrations: (a) 21.09 mmol kg^{-1} TC·HCl, (b) 23.06 mmol kg^{-1} TC·HCl, (c) 25.19 mmol kg^{-1} TC·HCl and (d) 27.08 mmol kg^{-1} TC·HCl.

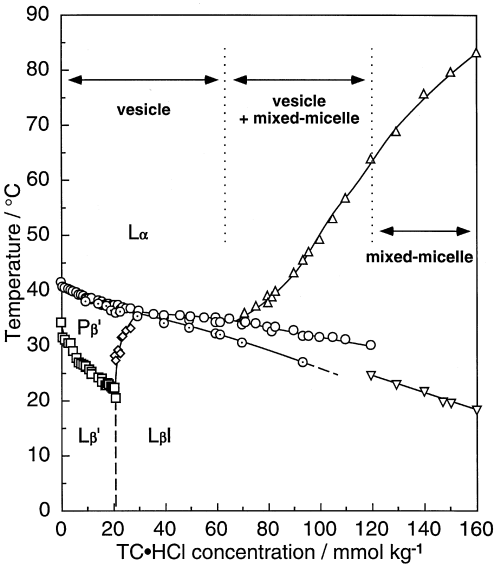

Fig. 4. Phase transition temperatures of DPPC bilayer membrane as a function of TC·HCl concentration. Phase transition: (□) pre-transition, (◇) $L_\beta I \rightarrow P_\beta'$ transition, (○) main transition, (⊙) main transition on the cooling scan, (△) new transition 1, (▽) new transition 2. DPPC concentration is 2.0 mmol kg^{-1}.

DPPC bilayer membrane. On the other hand, the thermodynamic equation expressing the depression of the phase-transition temperature includes the transition enthalpy (ΔH_t) in denominator [9,20]. Since the enthalpy change for the pretransition is significantly smaller than that for the main transition [2–5], the depression of the transition temperature for the pretransition is amplified. It is noteworthy that the $L_\beta I$ phase instead of the P_β' phase appears at a concentration of TC·HCl above 21 mmol kg^{-1}. Interdigitation of the DPPC bilayer has been observed by the addition of ethanol [14,21–24], 1-butanol [38] and other surface-active small molecules [25,39,40], and induced by the high-pressure [28–33]. The temperature of transition from the $L_\beta I$ phase to the P_β' phase showed a steep increase with an increase in TC·HCl concentration, then the transition was absorbed into the main transition in the presence of TC·HCl above 28 mmol kg^{-1}. A phase boundary between the L_β' and the $L_\beta I$ phases was observed at a narrow range of TC·HCl concentration of approximately 21 mmol kg^{-1}. This TC·HCl concentration can be regarded as the critical concentration for the interdigitation of DPPC bilayer. Since the $L_\beta I$ phase was induced by both pressure and tetracaine, the induction of the $L_\beta I$ phase has been confirmed by the methods of high-pressure FT-IR [41] and high-pressure ^{31}P-NMR [26]. These observations were performed at a finite concentration of tetracaine. The present results, however, show the critical concentration of tetracaine for the interdigitation of the DPPC bilayer membrane. With respect to the phase boundary between L_α and $L_\beta I$ phases, there was the temperature gap between heating and cooling scans. Rowe and Cutrera [21] have observed similar hysteresis on the phase transition of distearoylphosphatidylcholine bilayer membrane in the presence of ethanol above 25 mg ml^{-1}. This hysteresis may be attributable to a different receptivity to tetracaine partitioning between L_α and $L_\beta I$ phases and to a slow transformation into the $L_\beta I$ phase.

With respect to the mechanism of the induction of the $L_\beta I$ phase, McIntosh et al. [25] have explained the following. When small amphiphilic molecules are located in the interfacial region of

gel state DPPC liposomes, they anchor to the interface by virtue of their polar moiety, with the non-polar part of the molecule intercalating between the rigid acyl chains. In the case of short amphiphilic molecules whose non-polar moieties are not as long as the DPPC hydrocarbon chains, this would potentially cause voids between chains in the bilayer interior. Since the energy of formation of holes in hydrocarbons is extremely large, the chains must eliminate the voids. To do this, the chains in the bilayer could interdigitate. The lowest energy phase is the interdigitated phase. Recently, Kinoshita and Yamazaki [42] have proposed a new hypothesis, which is based on the χ (chi) parameter, that is, an interaction energy parameter between the surface segments of DPPC bilayer and solvents. The main reason for the induction of L_βI phase is the decrease of the χ parameter between the segments of the terminal alkyl chain of the phospholipid and solvents by the addition of organic molecules.

The new transitions 1 and 2 were observed in the presence of TC·HCl more than 71 and 110 mmol kg^{-1}, respectively. TC·HCl is known to form a micelle by itself at concentrations above 128 mmol kg^{-1} [36,37]. As mentioned below, the presence of LC·HCl and PC·HCl, which do not form micelles at any concentration, did not show the new transitions 1 and 2. Therefore, the new transitions 1 and 2 would concern themselves with the mixed micelle of DPPC and micelle-forming anesthetics. The temperature of the new transition 1 was elevated steeply as the concentration of TC·HCl increases, whereas the temperature of the new transition 2 was depressed gradually.

The phase transition enthalpies of DPPC bilayer membranes are shown in Fig. 5 as a function of TC·HCl concentration up to 160 mmol kg^{-1}. Enthalpy changes associated with the pretransition and the main-transition in the absence of TC·HCl were found to be 1.1 and 8.6 kcal mol^{-1}, respectively, which are in good agreement with previously reported data [2–5]. The enthalpy change for the pretransition decreased with increasing TC·HCl concentration and then approached to zero at 21 mmol kg^{-1} TC·HCl, which corresponds to the disappearance of the pretransition. In the case of the addition of ethanol, similar results have been observed [21]. The enthalpy of the main transition was reduced by 1.5 kcal mol^{-1} with increasing TC·HCl concentration up to 21 mmol kg^{-1}, and then increased steeply with an increase in TC·HCl concentration from 21 to 25 mmol kg^{-1}. An increase in enthalpy amounts to 1.8 kcal mol^{-1}. In the concentration range of 25–70 mmol kg^{-1} TC·HCl, the main-transition enthalpy increased gradually from 8.9 to 9.6 kcal mol^{-1} with increasing TC·HCl concentration. With respect to the main transition, the phase change observed is different, below 21 mmol kg^{-1} and above 25 mmol kg^{-1} TC·HCl, which is the P_β' to L_α transition and the L_βI to L_α transition, respectively. The decrease in enthalpy with increasing TC·HCl concentration below 21 mmol kg^{-1} may represent the P_β' phase being destabilized by the addition of TC·HCl. In contrast, the appearance of the L_βI phase instead of the P_β' (or L_β') phase brings about the stabilization of gel phase by 1.8 kcal mol^{-1} and the L_βI phase is continuously stabilized by the addition of TC·HCl above 25 mmol kg^{-1}. The continuous addition of TC·HCl more than 70 mmol kg^{-1} brings about the drastic decrease in enthalpy of transition and the virtual disappearance of the main transition at the concentration above 140 mmol kg^{-1} TC·HCl. Judging from the ability of tetracaine to form micelles, we imagine the formation of DPPC–tetracaine mixed micelles and the vesicle-to-micelle transition induced by the addition of TC·HCl, which is discussed later on.

Second heating scans of DSC measurements were performed as follows. The DPPC–TC·HCl suspension was cooled to 5°C in the calorimeter sink after the first heating scan, allowed to stand for a short time (ordinarily 1–2 h), and then heated. The temperatures of the main transition between first and second scans were in good agreement with each other, but the enthalpy of the main transition showed hysteresis in the range of TC·HCl concentration from 80 to 140 mmol kg^{-1}, which is shown in Fig. 5 as closed circles. This hysteresis may be attributable to the mixed micelle-to-vesicle transformation being slow.

The enthalpy change for the new transition 1

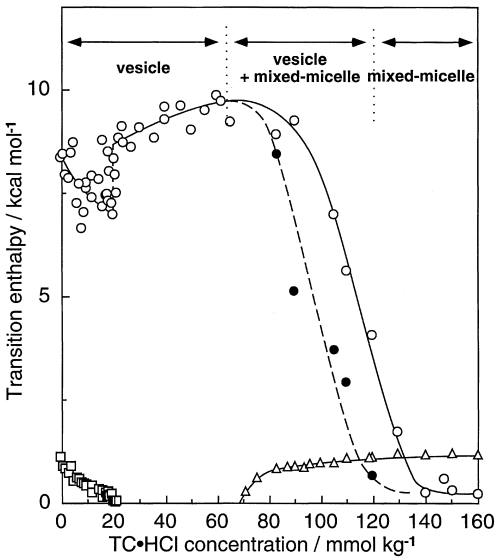

Fig. 5. Transition enthalpies of DPPC bilayer membranes as a function of TC·HCl concentration. Phase transition: (□) pre-transition, (○) main transition at the first scan, (●) main transition at the second scan, (△) new transition 1.

which appears in the presence of TC·HCl more than 71 mmol kg^{-1} increased with increasing TC·HCl concentration and remained at a fairly constant value at the concentration of TC·HCl above 90 mmol kg^{-1}. The enthalpy change for the new transition 2 cannot be determined because of the unstable baseline on DSC thermogram at lower temperature regions.

3.2. Effect of DC·HCl on DPPC bilayer membrane

The effect of DC·HCl on the phase-transition temperatures of DPPC bilayer membrane is shown in Fig. 6(A) as a function of DC·HCl concentration up to 90 mmol kg^{-1}. The situation of this phase diagram is almost the same as the DPPC–TC·HCl system (Fig. 4) except for the anesthetic concentration being lowered. This may be dependent upon the hydrophobicity of anesthetic molecules: dibucaine is more hydrophobic than tetracaine as is seen from the values of CMC for DC·HCl and TC·HCl to be 79 and 128 mmol kg^{-1} [36,37], respectively. The main transition of DPPC bilayer membrane was observed in all ranges of DC·HCl concentration studied although the endothermic peak became significantly smaller by the addition of DC·HCl over 60 mmol kg^{-1}. The addition of DC·HCl above the

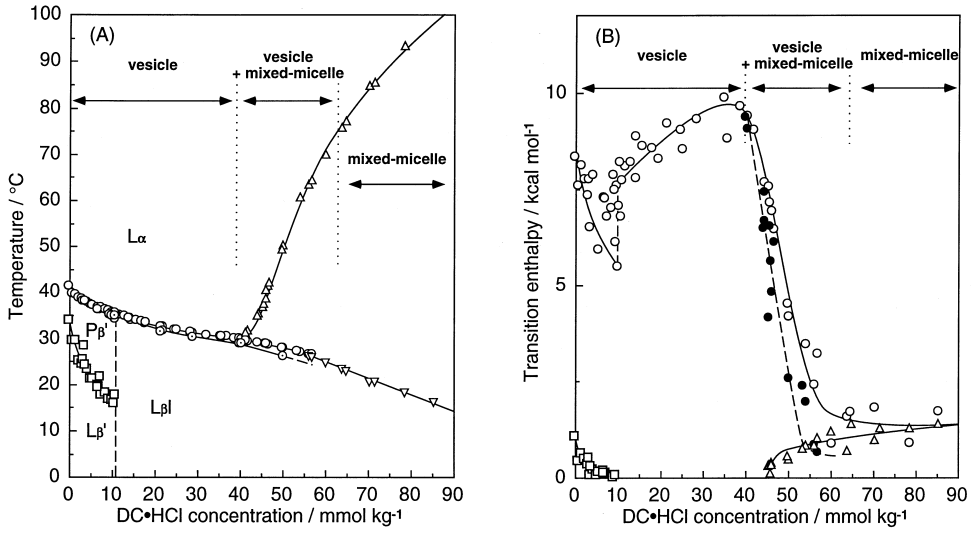

Fig. 6. Effect of DC·HCl on the DPPC bilayer membrane. (A) Transition temperature, (B) transition enthalpy. Phase transition: (□) pre-transition, (○) main transition, (⊙) main transition on the cooling scan, (●) main transition at the second scan, (△) new transition 1, (▽) new transition 2. DPPC concentration is 2.0 mmol kg^{-1}.

concentration of 11 mmol kg^{-1} brought about the disappearance of the pretransition and the induction of the interdigitation on the lipid bilayer membrane. The critical concentration of anesthetic-induced interdigitation was 11 mmol kg^{-1} for DC·HCl, which is lower than that (21 mmol kg^{-1}) for TC·HCl. The new transitions 1 and 2 were also observed in this system at the DC·HCl concentration above 42 and 56 mmol kg^{-1}, respectively. These transitions in the presence of dibucaine take place at lower anesthetic concentration than those in the presence of tetracaine, which are predictable from the difference in hydrophobicity of anesthetic molecules.

The effect of DC·HCl on the phase transition enthalpies of DPPC bilayer membranes is shown in Fig. 6(B) as a function of DC·HCl concentration up to 90 mmol kg^{-1}. The enthalpy profile of the DPPC–DC·HCl system resembles that of the DPPC–TC·HCl system. The enthalpy change for the pretransition exhibited a decrease with increasing DC·HCl concentration and then approached to zero in the presence of 11 mmol kg^{-1} DC·HCl at which the pretransition has disappeared. Regarding the main transition, the phase change being observed is different below and above 11 mmol kg^{-1} DC·HCl, which is the P_{β}' to L_{α} transition and the L_{β}I to L_{α} transition, respectively. The enthalpy of the main transition was reduced initially by 3.0 kcal mol^{-1} with an increase in DC·HCl concentration up to 11 mmol kg^{-1}, and then increased suddenly by 2.4 kcal mol^{-1} associated with the appearance of the L_{β}I phase. In the concentration range of 11–40 mmol kg^{-1} DC·HCl, the enthalpy of the L_{β}I to L_{α} transition increased gradually from 8.0 to 10.0 kcal mol^{-1} with increasing DC·HCl concentration. DC·HCl destabilized the P_{β}' phase and stabilized the L_{β}I phase. The effect of DC·HCl is similar to TC·HCl, but DC·HCl is more effective than TC·HCl. The addition of DC·HCl beyond 40 mmol kg^{-1} brings about the drastic decrease in enthalpy of transition in a similar manner to the DPPC–TC·HCl system. In the range of DC·HCl concentration of 40–60 mmol kg^{-1}, the main transition on DSC thermograms between first and second scans showed an enthalpy gap, which was observed already in the DPPC–TC·HCl system as mentioned before. The enthalpy change for the new transition 1 which appears in the presence of DC·HCl more than 42 mmol kg^{-1} increased gradually with increasing DC·HCl concentration.

3.3. Effects of LC·HCl and PC·HCl on DPPC bilayer membrane

The effect of LC·HCl on the phase-transition temperatures and the transition enthalpies of DPPC bilayer membrane are shown in Fig. 7 as a function of LC·HCl concentration up to 160 mmol kg^{-1}. With respect to the effect of PC·HCl, the phase-transition temperatures and the transition enthalpies are shown in Fig. 8 as a function of PC·HCl concentration up to 160 mmol kg^{-1}. Both the main transition and pretransition of DPPC bilayer membrane were observed throughout the anesthetic concentration studied. However, the L_{β}I phase and the new transitions 1 and 2 were not induced by these anesthetics. Both T_m and T_p were depressed gradually by the addition of LC·HCl or PC·HCl. All the anesthetics used depressed effectively T_p rather than T_m. The order of the depression of phase–transition temperatures by these local anesthetics was DC·HCl > TC·HCl > LC·HCl > PC·HCl, which was consistent with the order of hydrophobicity of anesthetic molecules. Lidocaine and procaine below 160 mmol kg^{-1} did not induce the L_{β}I phase in DPPC bilayer. Judging from the dependence of main- and pre-transition temperatures on their concentrations and the order of hydrophobicity of these local anesthetics, we presume that higher concentrations of lidocaine and procaine would induce the L_{β}I phase in the DPPC bilayer.

The enthalpy of the main transition of DPPC bilayer membrane decreased slightly by the addition of LC·HCl or PC·HCl. The difference in enthalpy decrease between both anesthetics was hardly detectable in the concentration range studied. With respect to the pretransition, the enthalpy decrease by the addition of LC·HCl is slightly significant compared with the addition of PC·HCl.

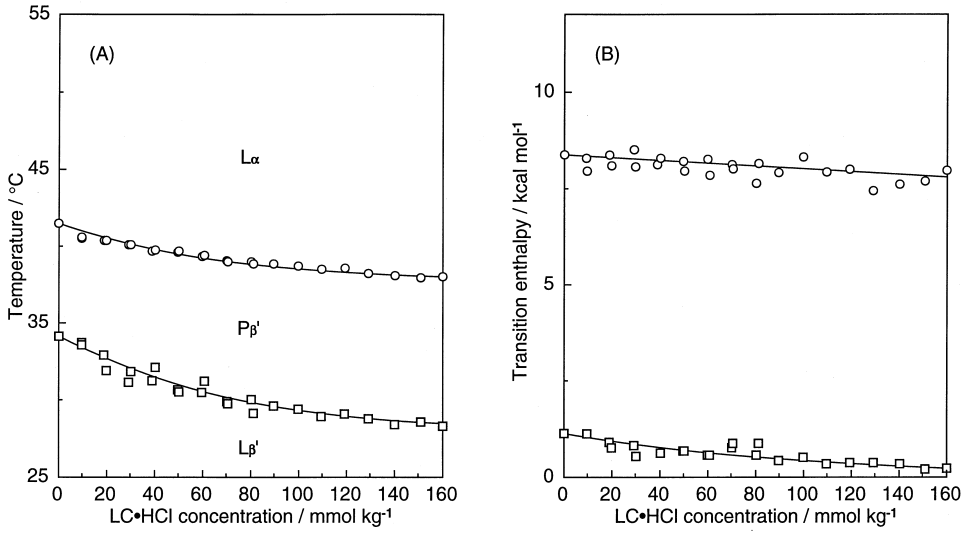

Fig. 7. Effect of LC·HCl on the DPPC bilayer membrane. (A) Transition temperature, (B) transition enthalpy. Phase transition: (□) pre-transition, (○) main transition. DPPC concentration is 2.0 mmol kg^{-1}.

3.4. Anesthetic induced vesicle-to-micelle transition

The studies of the vesicle–micelle transition in phospholipid–surfactant mixtures have been performed with various techniques including fluorescence [43–45], ESR [45], DSC [46], transmission electron microscopy [47,48], light scattering [47–50], HPLC [50] and small angle X-ray scattering [50]. These measurements have revealed the transformation pathway from vesicle to mixed micelle. Up to a certain concentration of surfactant, the vesicle size increases gradually due to swelling

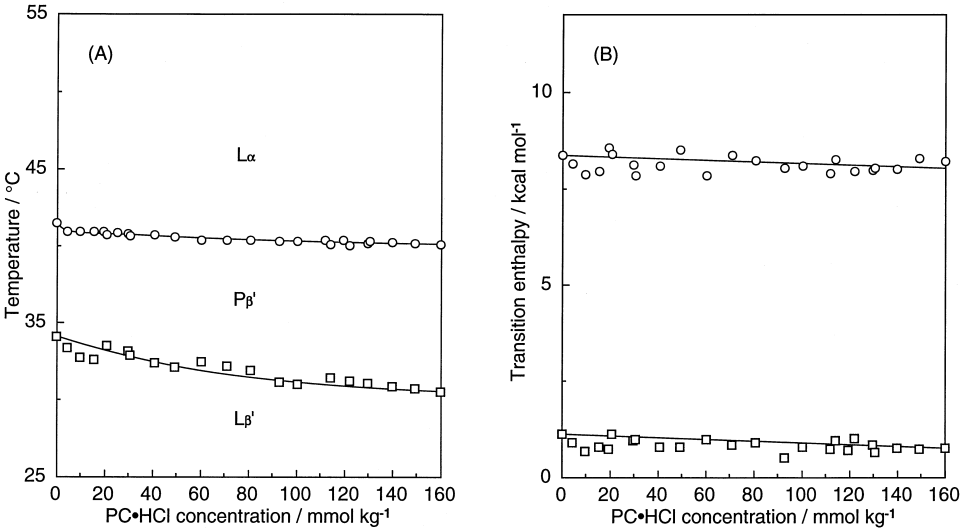

Fig. 8. Effect of PC·HCl on the DPPC bilayer membrane. (A) Transition temperature, (B) transition enthalpy. Phase transition: (□) pre-transition, (○) main transition. DPPC concentration is 2.0 mmol kg^{-1}.

Table 1
Average diameter (nm) of DPPC–TC·HCl aggregates in the solution at various concentrations of TC·HCl. DPPC concentration remains constant at 2.0 mmol kg^{-1}

Temperature	TC·HCl concentration (mmol kg^{-1})							
	0	60	70	80	90	100	120	140
45°C	321	292	282	212	88	34	< 5	< 5
35°C	312	301	269	241	70	27	< 5	< 5

of the bilayer membrane with the surfactant, regardless of the lipid concentration. At surfactant concentrations exceeding that point, the size of vesicle increases steeply followed by an abrupt drop when the lipid concentration is sufficiently high (ordinarily above 7 mmol kg^{-1}), whereas at a low concentration of lipids (below 2.5 mmol kg^{-1}) the size of vesicle decreases monotonously with an increase in the surfactant concentration.

Some local anesthetics behave as a surfactant because of the amphiphilic nature of anesthetic molecules, especially, TC·HCl and DC·HCl have the CMC of 128 and 79 mmol kg^{-1} [36,37]. In contrast, LC·HCl and PC·HCl do not have the ability to form micelle by themselves. Present results by DSC seem to reflect the difference in hydrophobic nature of local anesthetics. Therefore, phase behavior of DPPC bilayer membrane in the presence of TC·HCl or DC·HCl includes probably the vesicle–micelle transition. In order to confirm the occurrence of the vesicle–micelle transition, we measured the average size of vesicles by means of the dynamic light scattering as a function of TC·HCl concentration, which is summarized in Table 1. The average size of multilamellar vesicles remains fairly constant as a function of concentration up to 70 mmol kg^{-1} TC·HCl. Above this concentration the size of vesicles decreases monotonously with an increase in TC·HCl concentration, resulting in the mixed micelles above 120 mmol kg^{-1} TC·HCl. The present results could be superimposed on the drastic decrease in transition enthalpy at the TC·HCl concentration exceeding 70 mmol kg^{-1} which has been shown in Fig. 5. At the same time the new transition 1 has been observed at the concentration above 70 mmol kg^{-1} TC·HCl. Although the new transition 1 concerns itself with the mixed micelle, the change of phases for this transition is not assigned yet. In order to detect the aggregated species concerned with the mixed micelles,

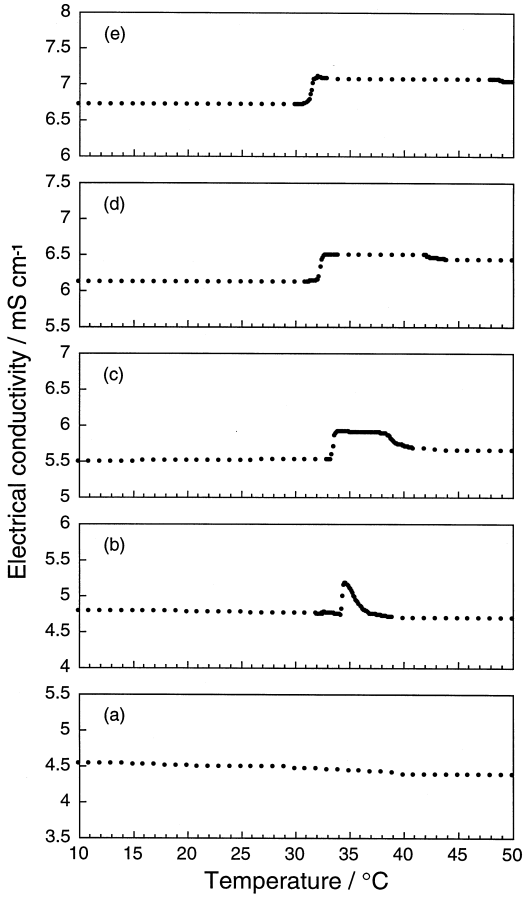

Fig. 9. Electrical conductivity of DPPC vesicle suspensions as a function of temperature at several TC·HCl concentrations: (a) 60.0 mmol kg^{-1} TC·HCl, (b) 70.0 mmol kg^{-1} TC·HCl, (c) 80.0 mmol kg^{-1} TC·HCl, (d) 90.0 mmol kg^{-1} TC·HCl and (e) 100.0 mmol kg^{-1} TC·HCl.

we measured the electrical conductivity of DPPC–TC·HCl mixed solutions at the same heating rate as the DSC scan. Fig. 9 shows the conductivity of DPPC suspensions in the presence of TC·HCl at several concentrations as a function of temperature. In the presence of TC·HCl below 60 mmol kg^{-1}, the conductivity of solutions did not change around the main-transition temperature. Contrarily the presence of TC·HCl above 70 mmol kg^{-1} brought about an abrupt increase in conductivity associated with the main transition and simultaneously the turbid solution became clear, and then the conductivity decrease at the temperature of new transition 1. Throughout the temperatures of between main transition and new transition 1, the conductivity of solution remains constant at the highest value. This rise in conductivity may be attributable to the formation of the DPPC–TC·HCl mixed micelles. Therefore, in the range of TC·HCl concentrations of 70–120 mmol kg^{-1} the DPPC vesicle solubilized TC·HCl coexists with the DPPC–TC·HCl mixed micelle, and the vesicle/micelle composition is dependent upon the concentration of TC·HCl. At the concentration of TC·HCl exceeding 120 mmol kg^{-1}, there exists the DPPC–TC·HCl mixed micelle. The concentration ranges coexisting with vesicle and mixed-micelle are shown in Figs. 4 and 5. The new transition 2, which was observed at the TC·HCl concentration above 120 mmol kg^{-1}, may be assigned as the phase transition of the DPPC–TC·HCl mixed micelle including the *trans–gauche* conformation change of lipid chains in the mixed micelle.

References

[1] H. Terada, Environ. Health Perspect 87 (1990) 213.
[2] S. Mabrey, J.M. Sturtevant, Proc. Natl. Acad. Sci. USA 73 (1976) 3862.
[3] M.C. Correa-Freire, E. Freire, Y. Barenhloz, P.L. Biltonen, Biochemistry 18 (1979) 442.
[4] J. Stümpel, A. Nicksch, H. Eibl, Biochemistry 20 (1981) 662.
[5] R.N.A.H. Lewis, N. Mak, R.N. McElhaney, Biochemistry 26 (1987) 6118.
[6] M.W. Hill, Biochim. Biophys. Acta 356 (1974) 117.
[7] I. Ueda, C. Tashiro, K. Arakawa, Anesthesiology 46 (1977) 327.
[8] A.G. Lee, Biochim. Biophys. Acta 514 (1978) 95.
[9] H. Kamaya, S. Kaneshina, I. Ueda, Biochim. Biophys. Acta 646 (1981) 135.
[10] E.S. Rowe, Biochemistry 22 (1983) 3299.
[11] Y. Kaminoh, C. Tashiro, H. Kamaya, I. Ueda, Biochim. Biophys. Acta 946 (1988) 63.
[12] T.J. O'Leary, P.D. Ross, I.W. Levin, Biophys. J. 50 (1986) 1053.
[13] J.A. Veiro, P. Nambi, L.L. Herold, E.S. Rowe, Biochim. Biophys. Acta 900 (1987) 230.
[14] P. Nambi, E.S. Rowe, T.J. McIntosh, Biochemistry 27 (1988) 9175.
[15] A.G. MacDonald, Biochim. Biophys. Acta 507 (1978) 26.
[16] D.B. Mountcastle, R.L. Biltonen, M.J. Halsey, Proc. Natl. Acad. Sci. USA 75 (1978) 4906.
[17] J.R. Trudell, D.G. Payan, J.H. Chin, E.N. Cohen, Proc. Natl. Acad. Sci. USA 72 (1975) 210.
[18] H. Kamaya, I. Ueda, P.S. Moore, H. Eyring, Biochim. Biophys. Acta 550 (1979) 131.
[19] W. MacNaughtan, A.G. MacDonald, Biochim. Biophys. Acta 597 (1980) 193.
[20] S. Kaneshina, H. Kamaya, I. Ueda, J. Colloid Interface Sci. 93 (1983) 215.
[21] E.S. Rowe, T.A. Cutrera, Biochemistry 29 (1990) 10398.
[22] K. Ohki, K. Tamura, I. Hatta, Biochim. Biophys. Acta 1028 (1990) 215.
[23] J. Zeng, P.L.-G. Chong, Biochemistry 30 (1991) 9485.
[24] S. Kaneshina, T. Tamura, H. Kawakami, H. Matsuki, Chem. Lett. (1992) 1963.
[25] T.J. McIntosh, R.V. McDaniel, S.A. Simon, Biochim. Biophys. Acta 731 (1983) 109.
[26] X. Peng, J. Jonas, Biochemistry 31 (1992) 6383.
[27] S. Maruyama, T. Hata, H. Matsuki, S. Kaneshina, Biochim. Biophys. Acta 1325 (1997) 272.
[28] L.F. Braganza, D.L. Worcester, Biochemistry 25 (1986) 2591.
[29] R. Winter, W.C. Pilgrim, Ber. Bunsenges. Phys. Chem. 93 (1989) 708.
[30] S.K. Prasad, R. Shashidhar, B.P. Gaber, S.C. Chandrasekhar, Chem. Phys. Lipids 43 (1987) 227.
[31] D.A. Driscoll, J. Jonas, A. Jonas, Chem. Phys. Lipids 58 (1991) 97.
[32] S. Maruyama, H. Matsuki, H. Ichimori, S. Kaneshina, Chem. Phys. Lipids 82 (1996) 125.
[33] H. Ichimori, T. Hata, H. Matsuki, S. Kaneshina, Biochim. Biophys. Acta 1414 (1998) 165.
[34] M.S. Fernandez, Biochim. Biophys. Acta 597 (1980) 83.
[35] D. Attwood, P. Fletcher, J. Pharm. Pharmacol. 38 (1986) 494.
[36] H. Satake, H. Matsuki, S. Kaneshina, Colloids Surf. A 71 (1993) 135.
[37] H. Matsuki, S. Hashimoto, S. Kaneshina, M. Yamanaka, Langmuir 10 (1994) 1882.
[38] F. Zhang, E.S. Rowe, Biochemistry 31 (1992) 2005.
[39] R.V. McDaniel, T.J. McIntosh, S.A. Simon, Biochim. Biophys. Acta 731 (1983) 97.
[40] S.A. Simon, T.J. McIntosh, Biochim. Biophys. Acta 731 (1984) 169.

[41] M. Auger, H.C. Jarrell, I.C.P. Smith, D.J. Siminovitch, H.H. Mantsch, P.T.T. Wong, Biochemistry 27 (1988) 6086.
[42] K. Kinoshita, M. Yamazaki, Biochim. Biophys. Acta 1284 (1996) 233.
[43] M. Ollivon, O. Eidelman, R. Blumenthal, A. Walter, Biochemistry 27 (1988) 1695.
[44] O. Eidelman, R. Blumenthal, A. Walter, Biochemistry 27 (1988) 2839.
[45] T. Inoue, H. Kawamura, S. Okukado, R. Shimozawa, J. Colloid Interface Sci. 168 (1994) 94.
[46] C.H. Spink, V. Lieto, E. Mereand, C. Pruden, Biochemistry 30 (1991) 5104.
[47] A. de la Maza, J.L. Parra, Langmuir 11 (1995) 2435.
[48] M. Silvander, G. Karlsson, K. Edwards, J. Colloid Interface Sci. 179 (1996) 104.
[49] M. Ueno, Y. Akechi, Chem. Lett. (1991) 1801.
[50] B. Carion-Taravella, J. Chopineau, M. Ollivon, S. Lesieur, Langmuir 14 (1998) 3767.