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Effects of Pressure and Ethanol on the Phase Behavior of Dipalmitoylphosphatidylcholine Multilamellar Vesicles

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The temperature-pressure phase diagrams of dipalmitoylphosphatidylcholine multilamellar vesicles were constructed in the presence of ethanol. Pressure- and/or ethanol-induced gel phase was observed. Pressure facilitates, rather than antagonizes, the effect of ethanol on the occurrence of interdigitated gel phase in lipid bilayers.

Pressure studies of lipid bilayer membranes have been initiated in the interest of a more complete understanding of pressure-anesthetic antagonism. The main phase transition temperatures of phospholipid bilayers are elevated by pressures and depressed by anesthetics.1) The succeeding high-pressure studies have been performed with various physical techniques including volumetry,2) X-ray diffraction,3) Raman spectroscopy,4) neutron diffraction,5) light transmission,6) and NMR.7) These measurements have revealed phase behaviors of dipalmitoylphosphatidylcholine (DPPC) bilayers. In addition to liquid crystal, ripple gel and lamellar gel phases, a new pressure-induced gel phase, in which the lipid acyl chains from opposing monolayers are fully interdigitated, was observed.5-7)

On the other hand, ethanol is known to have a biphasic effect on the main transition temperature of DPPC8) which is subsequently shown to be caused by induction of interdigitation in DPPC by ethanol.9) A temperature/ethanol phase diagram for DPPC has been determined by a variety of methods.10-12) Recently, both effects of pressure and ethanol on the DPPC bilayer membranes have been studied;13,14) however, phase behaviors in the presence of ethanol are still unknown.

The present study demonstrates the phase diagram of DPPC multilamellar vesicles (MLV) in the presence of ethanol by the method of high-pressure light transmission, and reveals the interaction between pressure and ethanol on the induction of interdigitated phase.

Synthetic DPPC was obtained from Sigma. Ethanol(Wako) was analytical grade. Water was purified by triple distillation. MLV was prepared by suspending DPPC in water at 2.5×10^{-3} mol·dm^{-3}, using a Branson Sonifier Model 185 and a cup horn. Ethanol was added to the MLV with microsyringes so as to be 0.4 mol·dm^{-3}. The phase transition temperatures were determined by observing the transmittance at a given pressure during the course of heating with scanning rate of 0.5 K·min^{-1}. The general arrangement of the high pressure apparatus has been described previously.15)

An example of the phase transition measurements under various pressures is depicted in Fig. 1. The sudden change of the transmittance accompanying the phase transition was followed at 540 nm. The heating scan at 0.1 MPa showed two kinds of transitions. Lower-temperature transition, so-called pretransition, refers to the transition from lamellar gel(L_β') to ripple gel (P_β') phase. On the other hand, higher-temperature transition refers to the main transition from P_β' phase to liquid crystal(L_α) phase. The midpoint of abrupt change in transmittance was taken as the phase transition temperature. These two transition temperatures were in good agreement with previously reported data.1b,2) Under high pressures (90 and 150 MPa in Fig. 1), the L_β' phase was transformed to interdigitated gel(L_βI) phase around 30 °C. The transition from L_β' phase to L_βI phase is accompanied with an increase in turbidity, which may be caused by increased molecular packing in MLV. This observation agrees with the report that the specific volume of the L_βI phase is less than that of the L_β' phase in MLV of DPPC.12,16)

The transition temperatures are known to be almost independent of the DPPC concentration up to about 60 wt%.17) The temperature-pressure diagrams of the phase transitions for DPPC-water system in the absence and presence of ethanol are shown in Figs. 2 and 3, respectively. In the absence of ethanol (Fig. 2), we see a pressure induced phase beyond 100 MPa, which can be assigned as the L_βI phase. A triple point at about 100 MPa, 45 °C is in good agreement with previous data.6,7) As is seen from Fig. 2, the slope of the temperature-pressure diagram, dT/dP, for the phase boundary between L_β' and L_βI phases is negative, which means that the transformation from L_β' phase to L_βI phase is accompanied by the negative volume change in accordance with the Clausius-Clapeyron relationship.

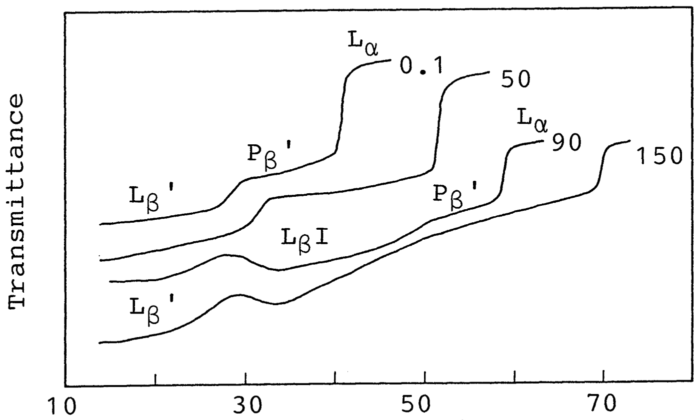

Fig. 1. Typical heating scans for transition measurements of DPPC bilayers in 0.4 M ethanol. Transmittance scale is arbitrary. Numerical values are pressure in MPa.

The value of dT/dP for the main transition and pretransition were 0.244 and 0.140 K•MPa^{-1}, respectively, which are comparable to those in literatures.[1b,2,6]

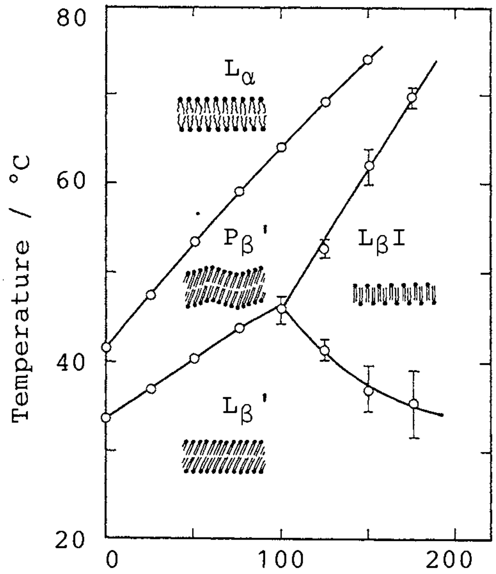

Fig. 2. Phase diagram for MLV of DPPC. Bilayer structures are schematically shown.

Figure 3 shows the phase diagram of DPPC bilayers in the presence of 0.4 mol•dm^{-3} ethanol. The L_β'-$L_\beta I$-P_β' triple point was observed at 50 MPa, 32 °C, and the $L_\beta I$-P_β'-L_α triple point was at 125 MPa, 65 °C. The interdigitated phase appeared at the pressure above 50 MPa, whereas the ripple gel phase disappeared at the pressure above 125 MPa. The phase boundary between L_β' phase and $L_\beta I$ phase was transformed from negative slope to positive slope by the pressure above 100 MPa. This pressure reversal of phase boundary slope can be accounted for by the larger compressibility of L_β' phase. Consequently, the specific volume of the $L_\beta I$ phase in the presence of ethanol is probably larger than that of the L_β' phase at the pressure above 100 MPa. It is obvious from two phase diagrams (Figs. 2 and 3) that ethanol depresses effectively the pretransition temperature rather than the main transition temperature under high pressures as well as ambient pressure, and the interdigitated phase extends the range to lower pressures by the addition of ethanol.

The interdigitated gel phase can be induced by a variety of small amphiphilic molecules such as benzyl alcohol, tetracaine, chloropromazine, methanol and glycerol besides ethanol.[18] These amphiphilic molecules anchor to the interface of bilayers by virtue of their polar moiety, with the non-polar part of the molecule intercalating between the lipid acyl chains. The lipid bilayers respond to the addition of amphiphilic molecules by forming the interdigitated phase, resulting in the decrease in the bilayer volume.[12] The interdigitated gel phase can be also induced by high pressure itself.[5-7] Since the volume of a system is decreased by the pressure, high pressure assists ethanol in forming the interdigitated gel phase in DPPC bilayers. On increasing the ethanol concentration in DPPC suspensions, the interdigitated gel

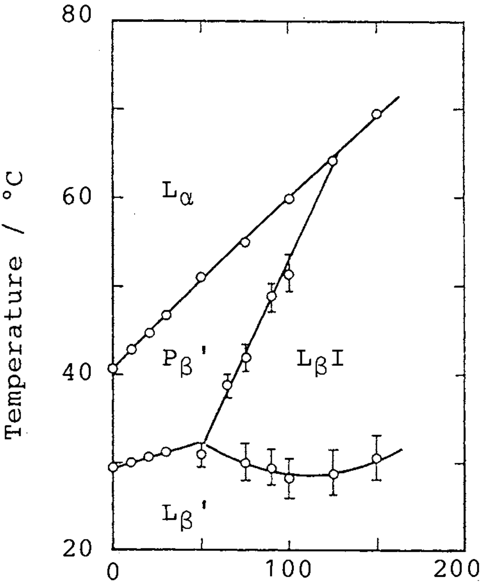

Fig. 3. Phase diagram of DPPC in the presence of 0.4 M ethanol.

phase should appear at lower pressure. Progressive addition of ethanol beyond 1 mol·dm^{-3} leads to finally the appearance of the interdigitated gel phase at ambient pressure.8) Although the interdigitated gel phase is evidently induced by both ethanol and pressure, the induction mechanism is not essentially the same. Both induction, however, bring about a common phenomenon which is the decrease in the bilayer volume. Consequently, the effects of pressure and ethanol are synergetic to the occurrence of interdigitated gel phase.

It is well known that ethanol causes anesthesia and pressure antagonizes ethanol-induced anesthesia. According to the molecular mechanism of anesthesia, pressure reversal of anesthesia may be caused by the increase in membrane order and/or the displacement of anesthetics from the membrane.1f,19) Present results revealed that pressure facilitates, rather than antagonizes, the effect of ethanol on the occurrence of interdigitated gel phases, which agrees with the observation by Prodan fluorescence.14) Therefore, we can conclude that the pressure-anesthetic antagonism must not originate from lipid interdigitation.

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