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Effect of Compound 48/80 on the Phase Transition Temperature and Permeability of Liposomal Membrane

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The action of compound 48/80 on liposomes prepared with dipalmitoylphosphatidylcholine (DPPC) and dicetylphosphate (DCP) was studied by means of fluorescence polarization and K^+ permeability measurements. Compound 48/80 interacted preferentially with DCP to decrease the phase transition temperature (T_c) of the membrane lipids. The K^+ release induced by 48/80 was temperature-dependent. It is proposed that the interaction between 48/80 and DCP forms a fluid-like domain in a rigid membrane matrix, resulting in the K^+ release from the liposomes.

Keywords——compound 48/80; membrane permeability; liposome; ion-selective electrode; K^+ marker; fluorescence polarization; phase transition

Compound 48/80 (a mixture of condensation products of *p*-methoxy-*N*-methylphenethylamine with formaldehyde) is widely used as a drug to cause histamine release from mast cells.[1] We have also been studying the action of 48/80 on liposomal membranes, and recently reported that this compound has a high affinity to acidic phospholipid and induces a permeability change of liposomal membrane.[2] It has been proposed that 48/80 causes a significant molecular disorder in bilayer structures, and thus results in an increase of the permeability of lipid membrane. This paper discusses the correlation between the permeability change and the change in phase transition temperature (T_c) of the liposomes upon addition of 48/80.

Materials and Methods

Materials——Dipalmitoylphosphatidylcholine (DPPC), dicetylphosphate (DCP), and compound 48/80 were purchased from Sigma Chemical Co. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was a product of Tokyo Kasei Kogyo Co., Ltd. Other chemicals used were all of analytical reagent grade.

Methods——The fluorescence polarization technique was used for the measurement of phase transition temperature (T_c) in sonicated liposomes.[3] The lipids together with the fluorescence probe (DPH, 1 mol% of the lipids) were dispersed in 0.15 M NaCl and 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)-NaOH (pH 7.4), and then ultrasonicated (Bransonic B-220, 125W) at a temperature above T_c until a clear preparation was obtained (about 2—3 min). The final lipid concentration was adjusted to 0.1 μmol/ml, and 48/80 was added subsequently (usually 20 μg/ml). The measurements were carried out on a Hitachi MPF-4 fluorospectrophotometer equipped with thermoregulated cells. The degree of polarization was calculated according to the following equation:

$$P=\frac{I_{VV}-C_f I_{VH}}{I_{VV}+C_f I_{VH}}$$

where I is the fluorescence intensity and subscripts V and H refer to the vertical and horizontal orientations of the excitation (first) and emission (second) polarizers, respectively. C_f ($=I_{HV}/I_{HH}$) is a correction factor.[4] The temperature in the cell was determined using a thermistor.

Permeability measurements were carried out to determine K^+ leakage from K^+-entrapped multilamellar liposomes by using a K^+ ion-selective electrode as previously described.[2,5]

Results and Discussion

Figure 1 shows the effect of 48/80 on the phase transition temperature (T_c) of DCP membranes.[6] The mid-temperature of the phase transition in a polarization experiment, such as that shown in Fig. 1, is defined as T_c.[3] A remarkable decrease of T_c was observed after addition of 48/80 to the lipid suspension. In lipid mixtures of DPPC and DCP, 48/80 also induced a lowering of T_c; however, as the ratio of DPPC was increased the effect of 48/80 was markedly reduced (Fig. 2). These results imply that 48/80 interacts preferentially with acidic phospholipid and increases the fluidity of the lipid bilayer.

The decrease of T_c induced by 48/80 was considered to arise as follows. Since 48/80 is a polycation with appropriate lipophilicity, it interacts electrostatically with negatively charged lipid constituents in liposomes and its lipophilic portion can penetrate the lipid bilayer.[2] Although simple electrostatic interaction between a cationic substance and an acidic phospholipid will form a tight domain by neutralization of the lipid charge, resulting in a rise

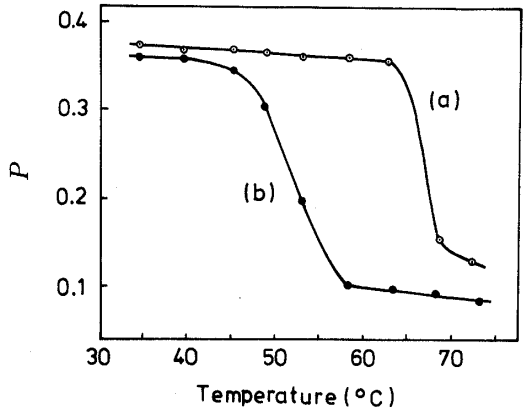

Fig. 1. Phase Transition Change of DCP Membranes before and after Addition of 48/80

(a) DCP alone (0.1 μmol/ml). (b) After addition of 48/80 (20 μg/ml).

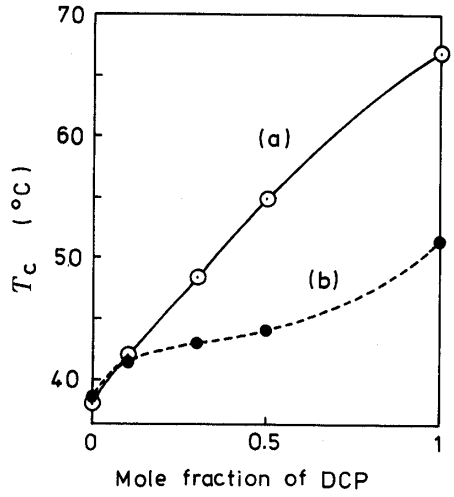

Fig. 2. Lowering of T_c of DPPC/DCP Liposomes by 48/80

T_c was estimated from the transition curves obtained by increasing the temperature. (a) Changes in T_c in DPPC/DCP mixtures at various ratios without 48/80. (b) T_c change after addition of 48/80 (20 μg/ml).

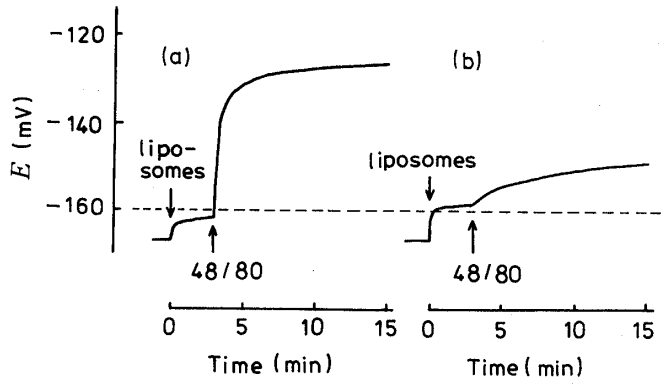

Fig. 3. Temperature Effect on the K^+ Release upon Addition of 48/80

At zero time, 0.2 ml of liposome suspension (containing 2 μmol of lipids) was added to 1 ml of 0.15 M NaCl and 5 mM Hepes–NaOH (pH 7.4). After 3 min, 0.1 ml of 48/80 (10 μg) was added. The measurements were performed at 25 °C (a) and at 9 °C (b).

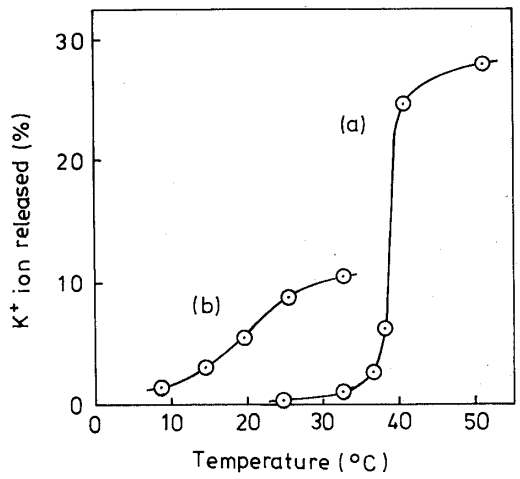

Fig. 4. Temperature Dependence of K^+ Release

The percentage of K^+ release was determined from the amount of K^+ efflux within 10 min. The total amount of the K^+ marker was determined by disrupting the liposomes with an ultrasonicator for 15 min at 55 °C. (a) DPPC/DCP mixture at a molar ratio of 1 : 0.1. (b) After addition of 48/80 (7.7 μg/ml).

of T_c, the penetration of the lipophilic portion into the membrane weakens the hydrophobic interaction between the phospholipid molecules and leads to an expansion of the lipid bilayer structure to decrease T_c.[7]

Next, we considered the correlation between the phase transition change and the change in the K^+ permeability upon addition of 48/80. The permeability change has been observed in liposomes prepared from DPPC and DCP in a molar ratio of 1 : 0.1.[2] Although these liposomes did not provide a marked lowering of T_c upon addition of 48/80 (Fig. 2), it seems likely that the interaction between 48/80 and DCP produces a local microscopic region in which lipid arrangement in the membrane is disordered. The formation of such a local microscopic disorder, which increases the membrane fluidity, might be hindered at a lower temperature.[8,9] Figure 3 shows the effect of temperature on the K^+ release induced by 48/80 from liposomes prepared with DPPC and DCP in a molar ratio of 1 : 0.1. A slight increase of the K^+ concentration in the medium can be ascribed to spontaneous release from the liposomes. A rapid efflux of K^+ observed at room temperature (25 °C) was markedly suppressed at lower temperature (9 °C). Figure 4 shows the K^+ release as a function of temperature in the presence and absence of 48/80. Without 48/80, the K^+ release was dramatically increased at *ca.* 40 °C, close to T_c of the liposomes (42 °C). The release of other markers such as Na^+ ion[8] and glucose[10] is also markedly augmented around T_c. It has been suggested that the phase boundary regions between the gel and liquid crystalline state significantly increase the membrane permeability.[8,9] The addition of polylysine (100 μg/ml) or Ca^{2+} (10 mM), which causes an increase of T_c of acidic phospholipid,[7] did not produce K^+ release from the present liposomes, indicating that the phase boundary in a rigid membrane does not provoke any permeability change. Accordingly, the K^+ release after addition of 48/80 is attributed to the formation of a fluid-like domain in the rigid membrane matrix, resulting in an increase of the membrane permeability of liposomes prepared with DPPC and DCP.

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