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Effect of Polyethylene Glycol Monoalkyl Ethers on Phase Transition Temperature of Dipalmitoylphosphatidylcholine Vesicle Membrane

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The gel-to-liquid-crystalline phase transition temperature of a dipalmitoylphosphatidylcholine vesicle membrane was measured in the presence of polyethylene glycol monoalkyl ethers (C_nE_m) of various chain lengths of hydrophobic (n) and hydrophilic (m) groups. All of the surfactants, except of $C_{12}E_1$, depressed the transition temperature almost linearly with the concentration. The depression of the phase-transition temperature was analyzed by applying the van't Hoff model for the freezing-point depression; also, the partition coefficients of the surfactants between bulk water and the membrane phase were estimated. Discussions were given concerning the dependence of the partition coefficients on the chain lengths of both oxyethylene and alkyl groups.

The interaction of surfactants with a phospholipid bilayer has been investigated in relation to the solubilization[1,2] or fusion[2-4] of biological membranes and to the preparation of lipid vesicles by detergent removal method.[5-7] Most of these studies deal with the mixed micelles formed by the addition of surfactants at a concentration higher than the critical micelle concentration. Recently, Goñi et al.[8] have paid attention to the effect of surfactants on the structure and properties of the lipid bilayer at rather lower concentration range. Such studies may have a significance different from the above-mentioned practical interest. Surfactants are typical amphiphiles, and the amphiphilicity can be readily controlled by combining various types of head groups and hydrocarbon tails. Hence, the surfactant-lipid bilayer system may provide a useful model system for us to understand the biologically significant interaction between amphiphilic ligands and a biological membrane.

Along the line of this interest, we have been studying the interaction of surfactants with model membranes in terms of their effect on the membrane properties.[9-11] As an extension of this series of studies, in the present work we investigated the effect of polyethylene glycol monoalkyl ethers, nonionic surfactants, on the gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine vesicle membrane. Interest was focused on the dependence of the effect on both the alkyl and oxyethylene chain lengths.

Experimental

Synthetic dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma. The following polyethylene glycol monoalkyl ethers with homogeneous chain lengths were used (abbreviated as C_nE_m, where n and m represent the carbon number of alkyl chain and the number of oxyethylene unit, respectively): n=10, m=6, 8; n=12, m=1, 3, 4, 5, 6, 7, 8; n=14, m=6, 8. All the surfactants were obtained from Nikko Chemicals Co.

A stock suspension of DPPC vesicle in water was prepared by sonication in the cup-horn of a Branson Sonifier Model 185 at 45 °C for about 30 min. The sample suspension was prepared by mixing the DPPC suspension and surfactant solution to give the desired surfactant concentration, and by sonicating again at 45 °C for about 5 min. The sizes of vesicles were estimated from quasielastic light-scattering measurements to be about 160 nm in diameter; taking into account the size and the phase-transition pattern,[12] these vesicles may be regarded as being multilamellar. The DPPC concentration was kept at about 5×10^{-4} M† throughout the experiments.

The gel-to-liquid-crystalline phase transition of a DPPC vesicle membrane was followed by the scattered-light intensity from the vesicle suspension. Details concerning the procedure have been described previously.[9] Typical traces of the scattered-light intensity with temperature are

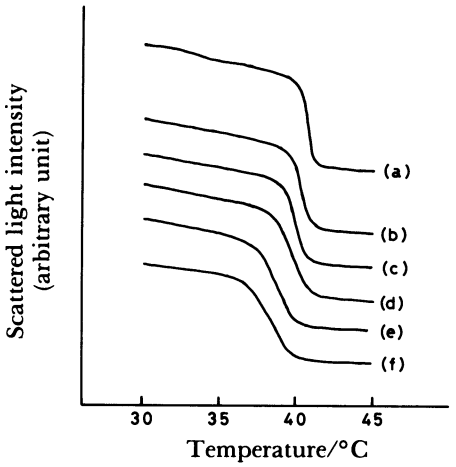

Fig. 1. Typical traces of the change in scattered light intensity at 400 nm with the temperature rise observed with DPPC-$C_{12}E_5$ system. Heating rate is 0.5 °C min^{-1}. DPPC concentration is 5.2×10^{-4} M. $C_{12}E_5$ concentrations (10^{-5} M) are (a) 0, (b) 2.52, (c) 3.37, (d) 5.05, (e) 6.73, and (f) 8.42.

† 1 M=1 mol dm^{-3}.

shown in Fig. 1. A drastic change in the scattered-light intensity was found corresponding to the phase transition. The transition temperature, T_m, was taken as the temperature at the half-height between two intersections of a line drawn through the steep portion of the transition region and the two lines drawn through the rather flat portions above and below the transition. The measurements were repeated at least three times for a given sample; a good reproducibility (typically within ±0.1 °C) was obtained for the transition temperature.

Results and Discussion

Figure 1 illustrates the effect of added $C_{12}E_5$ on the phase transition of a DPPC vesicle membrane. It can be seen that the transition temperature decreases with increasing $C_{12}E_5$ concentration. It was observed that at a higher concentration of the surfactant, the change in the scattered-light intensity at the transition region became small accompanied with the deformation of the transition curve; a further increase in the concentration led to an extremely low intensity of the scattered-light, probably due to the formation of a mixed micelle. This was also the case for other surfactants. Thus, the concentration ranges of added surfactants were limited to those where normal phase-transition patterns were observed.

Some examples of the relation between the transition temperature and the surfactant concentration are shown in Figs. 2a—c. In these figures, the depression of the transition temperature, $-\Delta T = -(T_m - T_{m0})$, is plotted against the surfactant concentration, where T_m and T_{m0} are the transition temperatures with and without the surfactants. As can be seen in these figures, $-\Delta T$ increases linearly with increasing added surfactant concentration.

The depression of the phase-transition temperature of lipid bilayers induced by additives of the small molecule has been successfully analyzed by applying the van't Hoff model for the freezing-point depression.[13-15] This model describes the transition temperature as being depressed as a result of the lowering of the chemical potential of lipid molecule in a liquid-crystalline phase due to the mixing with the additive molecules. According to this interpretation, ΔT is proportional to the mole fraction of additives in the lipid bilayer for the case of a sufficiently low additive concentration, which in turn allows an estimation of the partition coefficient of the additives between bulk water and a lipid bilayer when the additives are partitioned between these phases. Based on this treatment, ΔT is related to the partition coefficient, K, by the following equation:[9]

$$-\Delta T = \frac{RT_{m0}^2}{\Delta H} \cdot \frac{K}{55.5 + C_L K} C_A^0, \qquad (1)$$

where ΔH is the enthalpy change associated with the phase transition, R the gas constant, and C_L and C_A^0 the total concentrations of lipid and additives in molarity scale, respectively. K is defined as $K = x_A'/x_A$, where x_A' and x_A are the mole fractions of the additives in membrane and water phases, respectively.

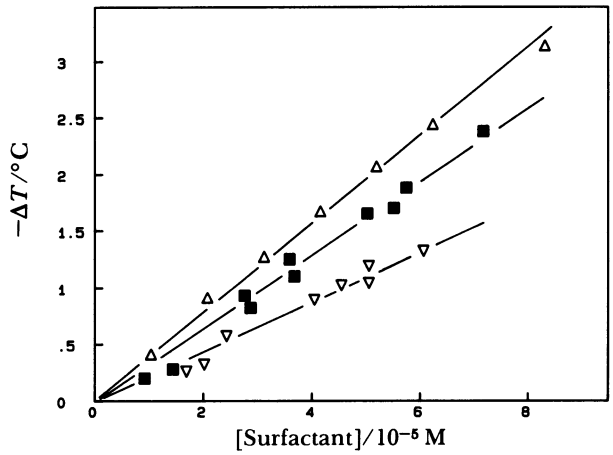

Fig. 2a. Plot of the depression of the phase transition temperature, $-\Delta T$, against the concentration of added surfactants. Surfactants are $C_{12}E_4$ (△), $C_{12}E_6$ (■), and $C_{12}E_8$ (▽).

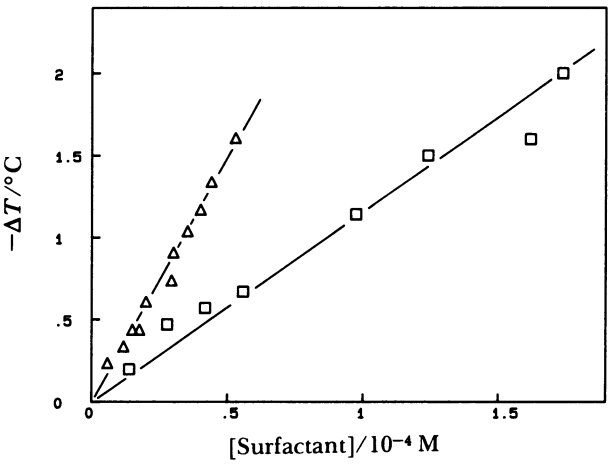

Fig. 2b. Plot of $-\Delta T$ against the added concentration of $C_{10}E_6$ (□) and $C_{14}E_6$ (△).

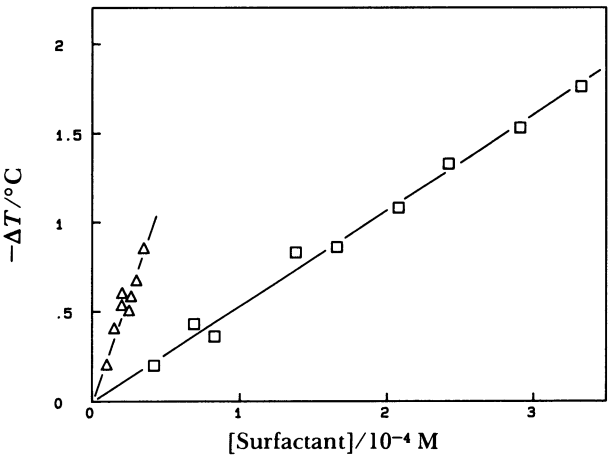

Fig. 2c. Plot of $-\Delta T$ against the added concentration of $C_{10}E_8$ (□) and $C_{14}E_8$ (△).

Equation 1 predicts a linear relationship between $-\Delta T$ and C_A^0, and allows an evaluation of the partition coefficient, K, from the slope. The partition coefficients of C_nE_m were estimated from the slopes of straight lines, as shown in Figs. 2a—c, using the values of T_{m0}=314 K and ΔH=36.4 kJ mol^{-1}.[16] Below, we examine the dependence of these K values on the oxyethylene chain length and the alkyl chain length.

Dependence of K on the Oxyethylene Chain Length. In Fig. 3, log K obtained with $C_{12}E_m$ is plotted against the number of oxyethylene unit, m. It can be seen that log K decreases almost linearly with increasing m in the range from m=4 to 8. The slope of this straight line provides $0.46RT$ as the contribution of an oxyethylene unit to the standard free energy change for the transfer of the surfactants from bulk water to the lipid bilayer. The critical micelle concentration (cmc)[17] of $C_{12}E_m$ is also shown in Fig. 3 for comparison. log cmc exhibits very weak dependence on m. This means that the contribution of oxyethylene unit to the standard free energy of micelle formation is negligibly small. For DPPC–surfactant systems previously studied,[9,11] there observed a good correlation between K and cmc; log K vs. log cmc plot gives straight lines with the slope of -1, the intercept of which weakely depends on the type of hydrophilic group. Comparing the present results for $C_{12}E_m$ with those for other surfactant systems, it is a wonder why the effect of oxyethylene chain length appears only in the partitioning into the lipid bilayer but not in the micelle formation. It is reasonable to consider that when surfactant molecules are incorporated in the lipid membrane, the hydrophilic head group of the surfactant is anchored at the membrane surface and the alkyl chain intercalates between the acyl chains of the lipid. The difference in the dependence on the oxyethylene chain length between K and cmc may be ascribed to a difference in the environment between the bilayer surface and the micellar surface.

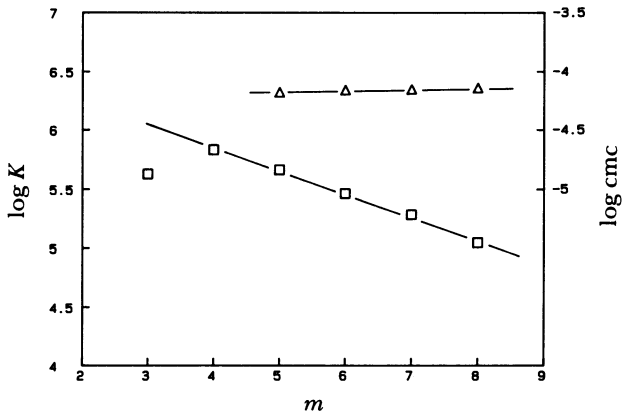

Fig. 3. Plot of log K (□, left scale) and log cmc (△, right scale) against the number of oxyethylene unit, m, for $C_{12}E_m$.

Considering the fact that the interior of lipid bilayer consists of the hydrocarbon region, it is of interest to compare the present partition data between water and lipid bilayer with those between water and hydrocarbon. Some partition data for nonionic surfactants with an oxyethylene chain between water and the hydrocarbon media are available in the literature.[18–20] From values of the partition coefficients, the free energies of the transfer from water to isooctane[18] and from water to cyclohexane[19,20] per oxyethylene unit are estimated to be $1.08RT$ and $0.96RT$, respectively. These are larger by a factor of about 2 than those of the transfer from water to DPPC bilayer. This difference may be attributed to the environmental difference between the partitions into the bulk hydrocarbon (water–hydrocarbon system) and into the water/hydrocarbon interface (water-bilayer system). The oxyethylene chain of the surfactants incorporated in the lipid bilayer locates at the bilayer surface and may be exposed to interfacial water; hence, the free energy loss may become less compared with the partition into the bulk hydrocarbon.

Another remarkable feature in Fig. 3 is the downward deviation of $C_{12}E_3$ from a straight line. This may be interpreted as follows. Equation 1 is based on the assumption that the amount of additives partitioned into the gel state bilayer is much smaller than those into the liquid-crystalline state, or even though the additives are incorporated substantially in gel phase, they are not randomly mixed but dispersed as aggregates (phase separation). It is likely that when the hydrophilicity of additives is reduced, the affinity to gel state bilayer becomes substantial, and the partitioning and mixing in gel state membrane becomes appreciable. In this case, Eq. 1 gives an underestimate of the partition coefficient. It may be

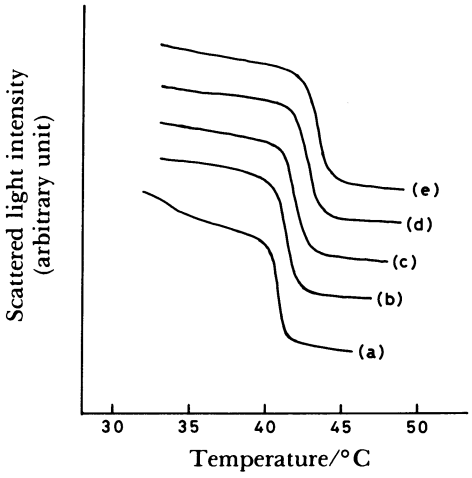

Fig. 4. Phase transition curves obtained with DPPC–$C_{12}E_1$ system. DPPC concentration is 5.0×10^{-4} M. $C_{12}E_1$ concentrations (10^{-4} M) are (a) 0, (b) 1.25, (c) 1.54, (d) 2.56, and (e) 3.42.

regarded that the K value obtained with $C_{12}E_3$ by applying Eq. 1 is not correct, but underestimated. When the oxyethylene chain length is further reduced, the effect of an additive on the phase transition becomes reversed; $C_{12}E_1$ elevated the transition temperature, as shown in Fig. 4. According to the van't Hoff model (described above) the elevation of the transition temperature can be interpreted as being due to the partitioning and mixing of the additives in the gel state bilayer, exceeding that in the liquid-crystalline state. A similar behavior of the additives concerning the phase-transition temperature of phosphatidylcholine vesicle membrane is well known for 1-alkanols; long-chain 1-alkanols with an alkyl chain length exceeding about 10 carbon atoms elevate the transition temperature, although shorter 1-alkanols depress it.[21-23] This suggests that the smaller hydrophilicity of 1-alkanols with longer alkyl chains favors their distribution in the gel state bilayer rather than liquid-crystalline state. The present results demonstrate that a similar reverse effect on the transition temperature is also induced by decreasing the hydrophilic oxyethylene chain length as the effect observed for 1-alkanol by increasing the hydrophobic chain length. Thus, the following general feature may be drawn for long-chain amphiphilic additives; reducing the hydrophilicity of the amphiphile below a certain critical level, it tends to partition into the gel state bilayer and mix with gel state lipids.

Dependence of K on the Alkyl Chain Length. Figure 5 shows the relation between log K and the carbon number of alkyl chain, N_c, obtained with C_nE_6 and C_nE_8. The increments of log K with N_c on going from 10 to 12 are similar for C_nE_6 and C_nE_8, which provide about 1 RT for the transfer free energy from the bulk water to the lipid bilayer per methylene unit. This increment of log K per methylene unit in alkyl chain is just the same as that obtained with other nonionic long-chain amphiphiles,[10] and the contribution of methylene unit to the transfer free energy is comparable to the free energy per methylene unit for the hydrophobic interaction.[24] On the contrary, from N_c=12 to 14, the increments of log K with N_c fall down for both C_nE_6 and C_nE_8, the extent of which is larger for C_nE_6 than C_nE_8. This deviation of log K for N_c=14 from the extraporated values from the lower N_c may be explained by the same way as the deviation of $C_{12}E_3$ from the linear relationship between log K and m in a series of $C_{12}E_m$ (Fig. 3). The hydrophilicity of $C_{14}E_6$ and $C_{14}E_8$ is weakened, relatively, due to their increased alkyl chain length, hence the partitioning and mixing in the gel state bilayer may occur substantially, and the K values obtained with these surfactants may be underestimated. The larger deviation for $C_{14}E_6$, whose hydrophilicity is less than $C_{14}E_8$, is consistent with this interpretation; i.e., the extent of underestimation of K is larger when the relative hydrophilicity is lower. A similar deviation of log K with increasing alkyl chain length was also observed for alkyltrimethylammonium salts.[9,11]

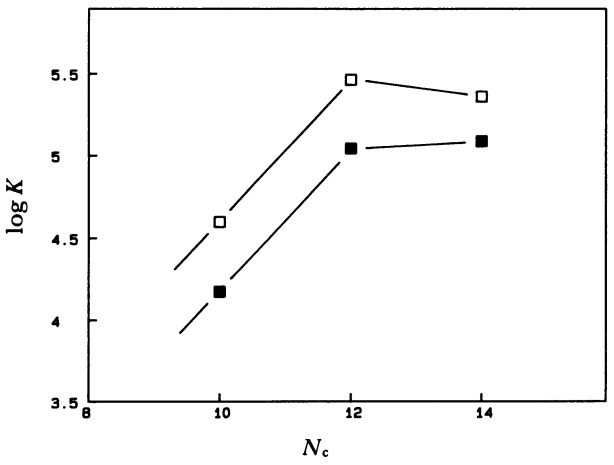

Fig. 5. Plot of log K against the carbon number in alkyl chain, N_c, for C_nE_6 (□) and C_nE_8 (■).

Conclusion

In this study, the partition coefficients of C_nE_m between bulk water and a DPPC bilayer were estimated from the depression of the phase transition temperature of a DPPC vesicle membrane by applying the van't Hoff model. The conclusion derived by examining the chain-length dependence of K is summarized as follows: (i) For a series of $C_{12}E_m$, log K decreased linearly with increasing m in the range between m=4 and 8. This suggests that the bilayer surface provides an energetically unfavorable fields for oxyethylene group compared with bulk water. (ii) $C_{12}E_3$ deviates from the linear plot of log K vs. m. $C_{14}E_6$ and $C_{14}E_8$ deviate from the linear plot of log K vs. N_c plot. These findings, along with the observation that $C_{12}E_1$ elevated the transition temperature, suggest that the decrease in the relative hydrophilicity of long-chain amphiphilic additives leads to partitioning into a gel state bilayer and mixing with the gel state lipids.

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