The Influence of Ureas on the Phase-Transition Behavior of the Dipalmitoylphosphatidylcholine Vesicle Membrane

Tohru INOUE,* Kenji MIYAKAWA,† and Ryosuke SHIMOZAWA
Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma, Jonan-ku, Fukuoka 814-01
†Department of Applied Physics, Faculty of Science, Fukuoka University, Nanakuma, Jonan-ku, Fukuoka 814-01
(Received April 13, 1987)

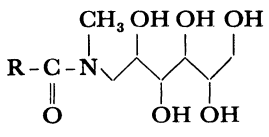
Synopsis. The gel-to-liquid-crystalline phase transition of the dipalmitoylphosphatidylcholine vesicle membrane was observed in the presence of urea, ethylurea, and butylurea by monitoring the scattered light intensity of the vesicle suspension. Urea showed no significant effect on either the transition temperature, T_m, or the transition width, W, whereas ethylurea and butylurea depressed T_m without affecting W.

In a previous work,[1] we studied the gel-to-liquid-crystalline phase transition of the dipalmitoylphosphatidylcholine (DPPC) vesicle membrane in the presence of surfactants with several types of head group and different hydrocarbon chain lengths. It was found that all the surfactants examined induced the depression of the phase-transition temperature, depending on the surfactant concentration. This depression of the transition temperature was interpreted in terms of the partitioning of the surfactant molecule into the lipid membrane.

In addition to the transition temperature, the transition width was also affected by the addition of surfactants. It was observed that surfactants other than the series of alkanoyl-*N*-methylglucamides (MEGA)†† increased the transition width and that the extent of the widening depended on the type of head group, but not on the hydrocarbon-chain length in a homologous series of surfactants.

The transition width is a measure of the cooperativity of the phase transition. The widening of the transition width induced by the addition of surfactants was interpreted as resulting from the fact that the surfactant molecules incorporated in the membrane tend to disrupt the interaction between lipid molecules responsible for the cooperativity. The origin of the cooperativity in the phase transition of the lipid bilayer has been attributed to the excluded volume interaction between hydrocarbon chains of lipid molecules.[2] However, the strong dependence of the transition width on the type of hydrophilic group of surfactants added suggests that the inter-head group interaction between lipid molecules also contributes to the cooperativity. Along the lines of this interpretation, it is of interest that MEGA's retain the transition width unaltered; i.e., MEGA's do not perturb the cooperativity. This feature may be attributed not to the electrical neutrality, but to the unique structure of the hydrophilic head group of these surfactants, because hexaethylene glycol monododecyl ether (nonionic surfactant),[1] 1-alkanols,[3,4] and uncharged anesthetic molecules[5,6] also exhibit a broadening effect on the phase transition. The hydrophilic group of MEGA contains both hydrogen-bond-donating and -accepting groups, which are lacking in other additives affecting the transition width. This led to the speculation that the hydrogen bond may play some role in the interaction associated with the cooperativity of the transition.

In order to test the above hypothesis, we have now studied the effects of urea and alkylurea on the phase-transition behavior of the DPPC vesicle membrane. These compounds are typical protein denaturants and have a strong tendency to form hydrogen bonds.

†† 

Experimental

The synthetic dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma. The ultrapure urea and reagent-grade ethylurea (Wako Pure Chemical Industries) and butylurea (99%, Aldrich) were used without further purification. The water was deionized and doubly distilled, once from an alkaline potassium permanganate solution.

The vesicle suspension of DPPC in water was prepared by sonication in the cup-horn of a Branson Sonifier, Model 185, at 45 °C (above the gel-to-liquid-crystalline phase-transition temperature) for about 30 min. The size of the vesicles thus prepared was estimated from the quasielastic light-scattering measurements to be about 160 nm in diameter; considering the size and the phase-transition pattern, these vesicles may be regarded as multilamellar. The sample suspension was prepared by mixing the stock DPPC suspension with the additive solution to give the desired additive concentration and by then sonicating it again at 45 °C for about 5 min. The DPPC concentration was kept at about 5×10^{-4} M††† throughout the experiments.

The gel-to-liquid-crystalline phase transition of the DPPC vesicle membrane was followed by means of the scattered light intensity. The details of the procedure were described elsewhere.[1] The measurements were repeated at least three times with a given sample; a good reproducibility was obtained for the transition temperature and the transition width.

Results and Discussion

The transition temperature, T_m, and the transition width, W, of the gel-to-liquid-crystalline phase transition of the DPPC vesicle membrane were measured as functions of the additive concentration. The transition temperature was expressed in terms of

††† 1 M=1 mol dm^{-3}.

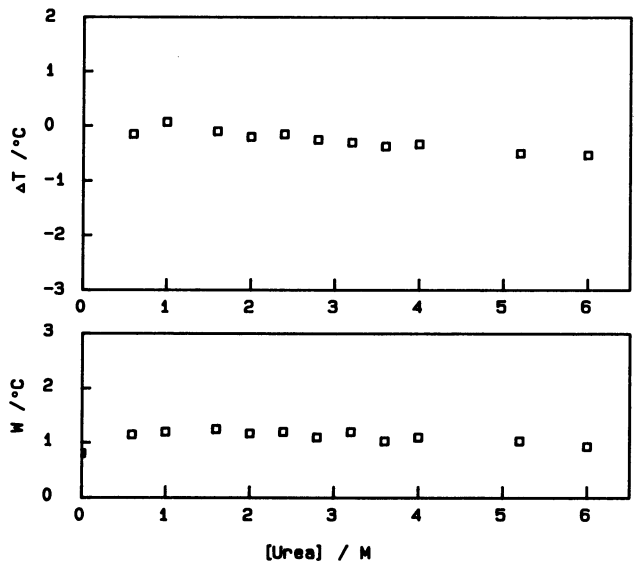


Fig. 1. Effect of urea on the transition temperature, ΔT, and transition width, W, of the gel to liquid-crystalline phase transition of DPPC vesicle membrane. $\Delta T=T_m-T_{m,0}$ where T_m and $T_{m,0}$ are the transition temperatures with and without additives, respectively.

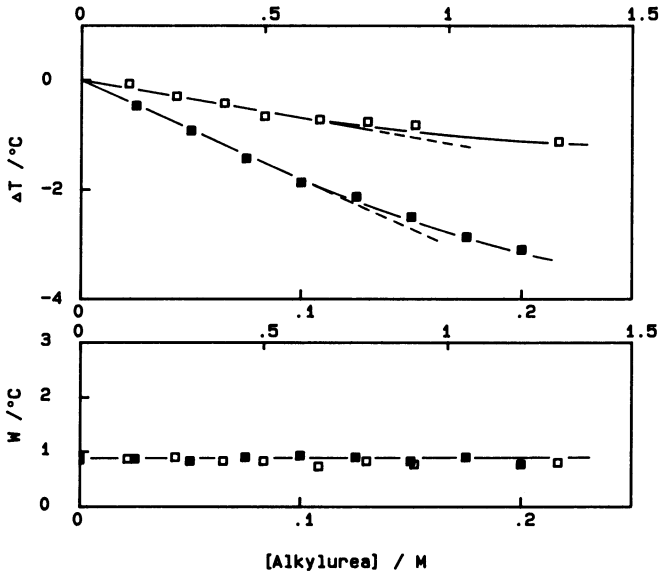


Fig. 2. Effect of alkylureas on ΔT and W of the gel to liquid-crystalline phase transition of DPPC vesicle membrane. □; ethylurea (upper scale) and ■; butylurea (lower scale).

the difference between $T_{m,0}$ (without additives) and T_m (with additives); i.e., $\Delta T=T_m-T_{m,0}$. The results obtained with urea are shown in Fig. 1. Neither T_m nor W is significantly affected by the presence of urea over a wide range of concentrations up to 6 M, although a slight decrease in T_m and a slight increase in W are seen. It is well-known that T_m is generally depressed by the dissolution of small molecular additives in the membrane as a result of an effect analogous to the freezing-point depression. The unaltered T_m suggests that urea is not incorporated into the lipid membrane.

It is believed that urea induces the breakdown of the water structure because of its strong tendency to form hydrogen bonds. Hammes and Schimmel[7] have demonstrated, on the basis of the ultrasonic absorption data in an aqueous urea solution, that the bulk water structure is broken down continuously with an increase in the urea concentration. The almost constant T_m and W values observed in DPPC-urea system indicate that the nature of the vesicle membrane is insensitive to the structure of bulk water with regard to the gel-to-liquid-crystalline phase transition.

The effects of ethylurea and butylurea on the phase-transition behavior are shown in Fig. 2. ΔT decreases linearly with an increase in the concentrations of the additives with the deviation from the linearity in the high-concentration region. On the other hand, W is unchanged by the presence of the additives. This behavior is just the same as that observed in studying the effects of the MEGA-series surfactants.[1]

Previously,[1] we have studied the effects of various surfactant molecules on the phase-transition behavior of the DPPC vesicle membrane; it was found that the transition width was dependent on the type of head group rather than on the hydrocarbon-chain length of the added surfactant. On the basis of this observation, and taking into account the fact that the head groups of surfactants may be located between head groups of DPPC when incorporated in the membrane, it was suggested that, in addition to the excluded volume interaction between hydrocarbon chains,[2] some other interaction between lipid head groups may participate in the cooperativity of the transition. MEGA series surfactants (Ref. 1) and alkylurea (this work) do not affect the transition width (or perturb the cooperativity). The common feature of both types of additives is that the hydrophilic group has a hydrogen-bond-donating group and -accepting group, which is lacking in other types of additives broadening the transition width (or weakening the cooperativity). If we assume that lipid head groups are connected throughout the membrane by the intermolecular hydrogen-bond-donating-accepting system, and that this system is responsible for the cooperativity of the transition, then it is plausible that the connection is broken and, hence, the cooperativity is weakened by introducing additives other than MEGA's or alkylureas. MEGA's and alkylureas may retain the hydrogen-bond system because of their hydrogen-bond-donating and -accepting nature. This may imply that the interaction between lipid head groups in the membrane is not disturbed by the presence of these additives and that, hence, the cooperativity is unaffected by the addition. A hydrogen bond associated with phosphatidylcholine has been reported recently,[8] where it was shown that hydrogen-bonding long-chain compounds form 2:1 complexes with DPPC when both hydrogen-bond-donating and -accepting groups are present.

The direct hydrogen bond between lipid head

groups is unlikely, considering the structure of phosphatidylcholine. It may be speculated that the hydrogen-bond interaction is mediated by water molecules present at the membrane surface. Indeed, it is regarded that the lipid head group in the bilayer dispersed in an aqueous media is fairly strongly hydrated:[9,10] 10—20 water molecules per lecithin molecule.

This work was supported in part by funds from the Central Research Institute of Fukuoka University.

References

1) T. Inoue, K. Miyakawa, and R. Shimozawa, *Chem. Phys. Lipids*, **42**, 261 (1986).
2) J. F. Nagle, *Ann. Rev. Phys. Chem.*, **31**, 157 (1980).
3) A. G. Lee, *Biochemistry*, **15**, 2448 (1976).
4) M. J. Pringle and K. W. Miller, *Biochemistry*, **18**, 3314 (1979).
5) D. M. Mountcastle, R. L. Biltonen, and M. J. Halsey, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 4906 (1978).
6) A. G. Macdonald, *Biochim. Biophys. Acta*, **507**, 26 (1978).
7) G. G. Hammes and P. R. Schimmel, *J. Am. Chem. Soc.*, **89**, 442 (1967).
8) J. M. Boggs, G. Rangaraj, and K. M. Koshy, *Chem. Phys. Lipids*, **40**, 23 (1986).
9) P. L. Yeagle, *Acc. Chem. Res.*, **11**, 321 (1978).
10) J. N. Israelachvili, S. Marcelja, and R. G. Horn, *Q. Rev. Biophys.*, **13**, 121 (1980).