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Effect of oligomers of ethylene glycol on thermotropic phase transition of dipalmitoylphosphatidylcholine multilamellar vesicles

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The effect of oligomers of ethylene glycol (EG) on thermotropic phase transitions of dipalmitoylglycerophosphatidylcholine multilamellar vesicles (DPPC-MLV) were investigated. Diethylene glycol (di-EG) had a biphasic effect on transition temperature, reducing pre-transition temperature (T_p) at low concentrations but increasing main transition temperature (T_m) and extinguishing pre-transition at high concentrations. Results of the X-ray diffraction method and the excimer method indicated that di-EG induced interdigitated gel phase ($L_{\beta I}$ phase) in the DPPC membranes at high concentration. Phase diagram of temperature-di-EG concentration for DPPC-MLV was determined by use of X-ray diffraction and differential scanning calorimetry, which was similar to that of temperature-EG concentration. The minimum concentration of di-EG where $L_{\beta I}$ phase was induced was 42%(w/v), which was larger than that of EG (30%(w/v)). On the other hand, in the presence of triethylene glycol (tri-EG), T_m and T_p increased with an increase in tri-EG concentration, as well as poly(ethylene glycol). These differences between the effects of di-EG and those of tri-EG, might be due to the differences of their sizes.

Introduction

Recently, interdigitated structures in phospholipid membranes have been studied and have attracted much attention [1]. In these structures, lipid molecules from opposing monolayers are fully interpenetrated or interdigitated and the terminals of the alkyl chains face the aqueous phase. Various alcohols, such as ethanol [2–4] and ethylene glycol (EG) [5], induce the interdigitated gel phase ($L_{\beta I}$ phase) in multilamellar vesicles (MLV) of phosphatidylcholine (PC). This type of interaction, between biomembranes and low molecular weight substances, is a new physical phenomenon.

We have recently proposed a novel concept of osmo-elastic coupling in biomembranes [6,7], which involves a new interaction of biomembranes with substances. In a suspension of phospholipid membranes in water, high molecular weight substances, such as poly (ethylene glycol) (PEG), are preferentially excluded from the region adjacent to the membrane surface (exclusion layer). On the other hand, small molecules, such as water or inorganic ions, are not excluded. Such exclusion causes the local imbalance of osmolarity between the exclusion layer and the bulk aqueous phase. As a result, it exerts osmotic stress on the vesicle membranes. At equilibrium, the membranes would be compressed to produce elastic pressure, which counterbalances the osmotic stress (osmo-elastic coupling). This concept can reasonably explain many phenomena observed in other biological systems [8–11].

We also have recently reported the effects of PEG and EG (monomer of PEG) on the thermotropic phase transitions of PC MLV [5]. Main transition temperature (T_m) and pre-transition temperature (T_p) of DMPC-, DPPC-, DSPC-MLVs increase with an increase in PEG 6000 concentration. These results were explained by enhancement of the lateral packing of the lipid on the basis of the osmo-elastic coupling theory [6,7]. On the other hand, EG induced the $L_{\beta I}$ phase in

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Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DMPC, 1,2-dimirystoyl-*sn*-glycero-3-phosphatidylcholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine; MLV, multilamellar vesicle; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); $L_{\beta'}$, tilted chain bilayer gel phase; $L_{\beta I}$, interdigitated gel phase; EG, ethylene glycol; di-EG, diethylene glycol; tri-EG, triethylene glycol; pyrene PC, 1-palmitoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphatidylcholine; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering; DSC, differential scanning calorimetry.

the membrane at high concentration. This fact indicates that the EG molecule can easily penetrate into the head group region of the lipid, in contrast with PEG 6000, because of the small size of the EG molecule.

From the above results, we can expect that larger water-soluble substances would exert more osmotic stress and less effectively induce the $L_{\beta I}$ phase in the membrane, due to the difficulty these molecules have penetrating into the headgroup region. In this brief communication, we investigate effects of oligomers of EG on thermotropic phase transitions of DPPC MLV. We choose oligomers of EG as water-soluble substances, since they have the same chemical properties. Their effects on the membrane may be only related to the size of their molecules. The most important question in this communication is to know the limiting size of oligomers of EG which induce the $L_{\beta I}$ phase.

A preliminary account of this research was presented at the Annual Meeting of the Physical Society of Japan, March, 1991, Tokyo.

Materials and Methods

Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) was purchased from Sigma. Diethylene glycol (di-EG) and triethylene glycol (tri-EG) were purchased from Wako. 1-Palmitoyl-2-pyrenedecanoyl-*sn*-glycero-2-phosphatidylcholine (pyrene PC) was purchased from Molecular Probes.

Methods

Multilamellar vesicles (MLV) were prepared by adding the appropriate amounts of various concentrations of di-EG or tri-EG solution in Pipes buffer (10 mM Pipes (pH 7.5), 140 mM NaCl) to the dry lipid. The mixture was then incubated for 10 min at 50°C. During the incubation time, the suspension was vortexed several times for 0.5 min. To attain equilibrium, the sample was incubated at 50°C for 1 h. After that, to complete equilibration of di-EG or tri-EG in the MLV, we sometimes used a freezing and thawing method [12]; samples were frozen in liquid N_2 for 1 min, removed to warm in the air (room temperature) for about 15 min to complete the thawing. The cycle of freezing and thawing was repeated ten times.

Differential scanning calorimetry (DSC) experiments have been described in detail before [5]. Phase transition temperature was determined at the onset of the endothermic transition extrapolated to the baseline. X-ray diffraction data were recorded using a position-sensitive proportional counter, as described before [5].

The excimer method was described in detail before [13]. The excitation wavelength of pyrene PC was 347 nm and emission wavelengths were 376 nm for

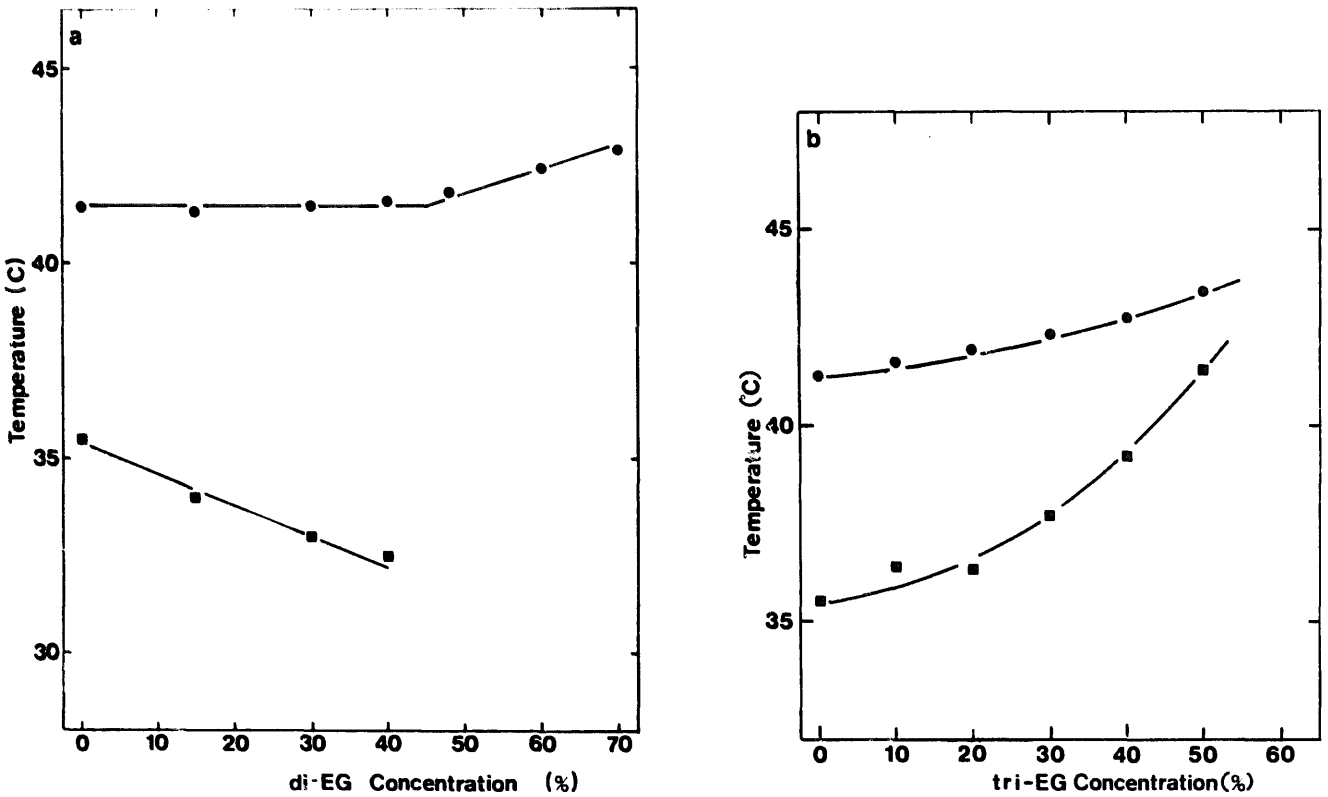

Fig. 1. Temperature of main transition (●) and pre-transition (■) of DPPC-MLV in the presence of di-EG (a) and tri-EG (b). Heating rates were 2 C°/min.

monomer fluorescence and 481 nm for excimer fluorescence. Samples for this fluorescence measurement were made as follows. Di-EG or tri-EG solution was added to the solution of the preformed MLV containing 3.3 mol% pyrene PC and the mixture was incubated at 50°C for 2 h and then the freezing and thawing of the samples was done six times.

Results and Discussion

As shown in Fig. 1, the results of the DSC measurements were different in the DPPC MLV in the presence of di-EG or tri-EG. In the interaction of DPPC MLV with di-EG, below 40%(w/v), the main transition temperature (T_m) was almost the same and pre-transition temperature (T_p) decreased with an increase in di-EG concentration, and above 48%(w/v) T_m increased slightly and there was no pre-transition peak (Fig. 1a). On the other hand, Fig. 1b shows the effects of tri-EG on the phase transitions of DPPC MLV. T_m and T_p increased with an increase in tri-EG concentration. This was the same result as the effect of PEG 6K [5].

The repeating period (spacing) of DPPC MLV measured by SAXS at 20°C increased slightly below 20%(w/v) and increased greatly in the range between 20%(w/v) and 50%(w/v), with increasing di-EG concentration, and then rapidly decreased from 80 Å to 64 Å at 50%(w/v) (Fig. 2). Wide angle reflection of DPPC MLV at 20°C was a single sharp reflection at 4.11 Å at 54%(w/v), showing the alkyl chains of the DPPC perpendicular to the bilayer surface. At 30%(w/v) and 40%(w/v), this consisted of a sharp reflection at 4.25 Å and a diffuse reflection centered at 4.1 Å, which is characteristic of the $L_{\beta'}$ phase [14]. A sudden and large decrease (16 Å) in the repeating period, together with the characteristic WAXS data, indicate that the MLV at high concentrations of di-EG was in the $L_{\beta I}$ phase, as discussed in the interaction of DPPC MLV with EG [5]. However, an electron density profile of DPPC MLV at high concentration of di-EG could not be determined, because there were no higher-order diffraction peaks than the 2nd order in the SAXS pattern. On the other hand, in the presence of tri-EG, the spacing of DPPC MLV at 20°C decreased slightly with an increase in tri-EG concentration (Fig. 2). The large increase of the spacing below 50%(w/v) of di-EG was due to the increase in the thickness of the water layer between the membranes inside MLV, because the WAXS data indicated that the angles of tilt of the alkyl chains of DPPC were almost the same and, thereby, the thicknesses of the membranes were almost the same below 50%(w/v). This increase in the thickness of the water layer may explain the fact that the spacing at 50%(w/v) di-EG was almost equal to that at the same concentration of tri-EG, in spite of the large decrease in the membrane thickness owing to the induction of the $L_{\beta I}$ phase in di-EG.

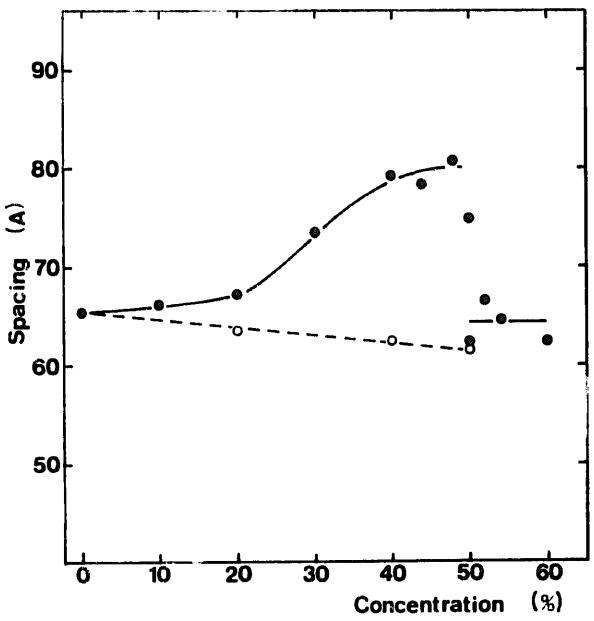

Fig. 2. Lamellar repeat period (spacing) of DPPC-MLV at 20°C at the various concentrations of di-EG (●) and tri-EG (○).

In order to confirm the induction of the $L_{\beta I}$ phase, the excimer method developed recently by us [13] was applied. The fluoresence intensity ratio E/M of pyrene PC in DPPC MLV was plotted as a function of di-EG concentration (Fig. 3). The E/M values at 17°C decreased sharply around 47%(w/v) of di-EG and above 55%(w/v) they were very small, which supports the induction of the $L_{\beta I}$ phase at high concentrations of di-EG. On the other hand, in the case of tri-EG (Fig. 3), E/M values were almost equal below 60%(w/v).

The temperature-di-EG concentration phase diagram for DPPC MLV was determined by use of the X-ray diffraction method (SAXS) and DSC method (Fig. 4). In SAXS, phase boundaries between $L_{\beta I}$ and $L_{\beta'}$ (or $P_{\beta'}$) were determined by monitoring the changes in the spacing measured by the strong first-order diffraction peak. For these measurements in SAXS, the temperature was increased in small steps (1 or 2 C°) at same concentration of di-EG after the equilibrium was attained [5]. This phase diagram can be divided into three regions. In the region of low concentration (region A, $C < 42\%$), the phase changes from $L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_{\alpha}$ as temperature increases. In the region of medium concentration (region B, $42\% < C < 45\%$), it changes from $L_{\beta'} \rightarrow L_{\beta I} \rightarrow P_{\beta'} \rightarrow L_{\alpha}$. In the region of high concentration (region C, $C > 45\%$), it changes from $L_{\beta'} \rightarrow L_{\beta I} \rightarrow L_{\alpha}$.

The temperature-dependence of wide angle reflection at 48%(w/v) di-EG was investigated. The wide-angle reflection at 28°C, 34°C, and 40°C showed single sharp reflections at 4.13 Å, 4.14 Å, and 4.16 Å, respec-

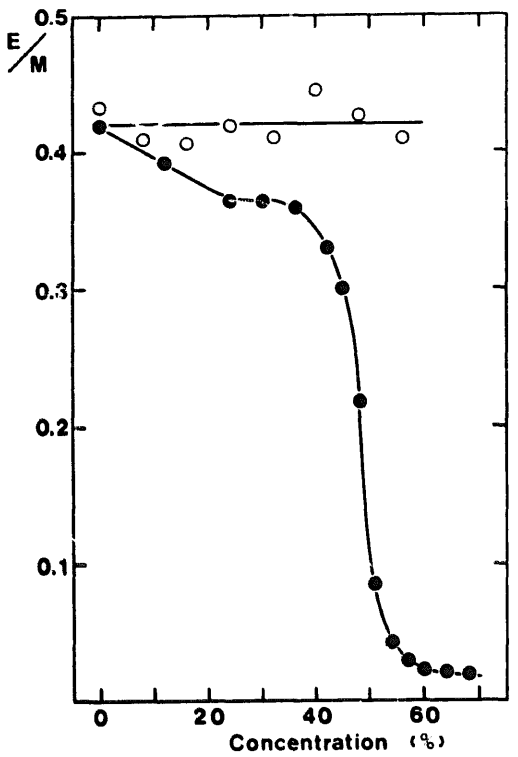

Fig. 3. Ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV at the various concentrations of di-EG (●) and tri-EG (○). E/M was determined at 17°C.

tively, which indicated that MLVs at these temperatures were in the $L_{\beta I}$ phase, because of the perpendicular orientation of the alkyl chains of the DPPC molecules to the bilayer surface. It also indicated that the distance of the alkyl chains in the $L_{\beta I}$ phase became slightly larger with increasing temperature. The

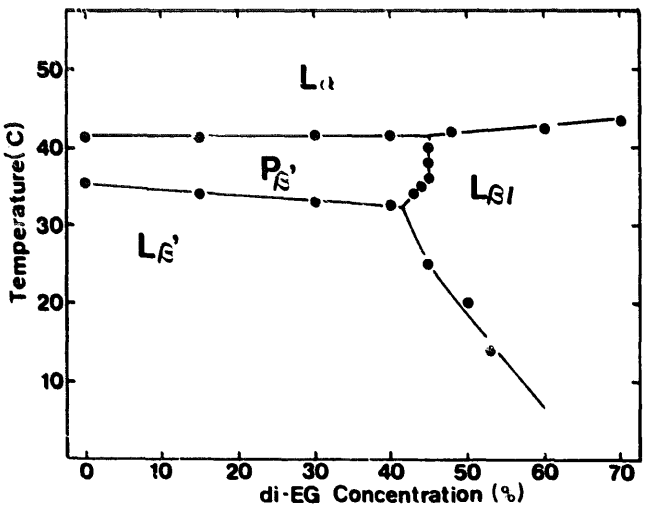

Fig. 4. Temperature-di-EG concentration phase diagram for DPPC-MLV. T_m and T_p were determined by the DSC method described in Fig. 1(a). The other transition temperatures were determined by SAXS.

wide-angle reflections at 10°C showing a sharp reflection at 4.22 Å, together with a diffuse reflection centered at 4.1 Å were characteristic of the $L_{\beta'}$ phase, where the alkyl chains of the DPPC molecules were tilted in the bilayer. These results supported the phase diagram (Fig. 4) determined by SAXS.

The results of the samples without freeze/thaw treatments (data not shown) were almost the same as those of the treated samples in the experiments of DSC and X-ray diffraction.

The results of X-ray diffraction methods and the excimer method indicated that, in the interaction of DPPC MLV with di-EG, the $L_{\beta I}$ phase was induced at high concentrations. Mechanisms for the induction of the $L_{\beta I}$ phase by low-molecular-weight substances can be considered as follows. In the absence of substances, the $L_{\beta'}$ phase is more stable than the $L_{\beta I}$ phase, because the hydrophobic terminal methyl group is exposed to water in the $L_{\beta I}$ phase and, thereby, the chemical potential of the lipid in the membrane of the $L_{\beta'}$ phase ($\mu(L_{\beta'})$) is lower than that of the $L_{\beta I}$ phase ($\mu(L_{\beta I})$). In the presence of substances such as ethanol, EG, and di-EG, these molecules penetrate into the head group region of the membrane. As a result, voids are formed in the bilayer interior [3] or, in some cases, the distances between the alkyl chains become wider, accompanying the distortion of the chain, which may increase $\mu(L_{\beta'})$. Moreover, such low-molecular substances play an important role in shielding the terminal methyl group in the $L_{\beta I}$ phase, which makes interfacial energy between the hydrophobic methyl group and solvent water lower [3,15], because it is well-known that the change of the standard chemical potential of alkane from water to alcohols such as ethanol and EG, is negative [16]. Such an effect may decrease $\mu(L_{\beta I})$. Therefore, above the critical concentrations of such substances, $\mu(L_{\beta I})$ becomes lower than $\mu(L_{\beta'})$ and, thereby, the $L_{\beta I}$ phase is induced. Hence, the formation of the $L_{\beta I}$ phase at high concentrations of di-EG is due to the easy penetration of di-EG into the head group region of the lipid in the gel phase MLV, owing to the small size of the di-EG molecule.

The phase diagram of the temperature-di-EG concentration for DPPC MLV was similar to that for EG [5] and ethanol [4]. The minimum concentration of di-EG where the $L_{\beta I}$ phase is induced was 42% (w/v), which is larger than that of EG (30%(w/v)). This difference could be explained as follows. Since the size of EG is smaller than that of di-EG, EG can more easily penetrate into the head group region of the lipid and, thereby, it can induce the $L_{\beta I}$ phase at lower concentration than di-EG.

On the other hand, in the presence of tri-EG, the T_m and T_p of DPPC-MLV increased with an increase in the tri-EG concentration, as in the presence of PEG [5]. This result could be explained as follows. Since the

size of tri-EG is larger than that of di-EG or EG, tri-EG molecules cannot penetrate into the head group region easily and may be preferentially excluded from the region adjacent to the membrane surface. Such preferential exclusion induces osmotic stress on the membranes and, at equilibrium, the membranes are compressed (osmo-elastic coupling). The increase of the phase transition temperatures induced by tri-EG could be explained by enhancement of the lateral packing of the lipids, as analyzed in the case of PEG [5].

To elucidate more clearly the mechanism of the difference between the effects of EG and di-EG or those of tri-EG, direct information of their effects on the structure and physical properties of the DPPC membrane is necessary. This problem will be treated in a following paper. In this brief communication, the following findings are most important: (1) oligomers of EG, which are smaller than and equal to di-EG, can induce the $L_{\beta I}$ phase in DPPC MLV and (2) the oligomers of EG which are larger than di-EG can increase the thermotropic phase transition temperature. These results might be extended to treat effects of other water-soluble low-molecular-weight substances on phase transition of PC membranes. That is, in the absence of specific interaction of the head group of the lipid with substances, smaller substances than the head group region could induce the $L_{\beta I}$ phase and larger substances than the headgroup region could increase the phase transition temperature. This new hypothesis must be verified by systematic experiments in the near future.

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References

1 Slater, J.L. and Huang, C.-H. (1988) Prog. Lipid Res. 27, 325–359.
2 Rowe, E.S. (1983) Biochemistry 22, 3299–3305.
3 Simon, S.A. and McIntosh, T.J. (1984) Biochim. Biophys. Acta 773, 169–172.
4 Ohki, K., Tamura, K. and Hatta, I. (1990) Biochim. Biophys. Acta 1028, 215–222.
5 Yamazaki, M., Ohshika, M., Kashiwagi, N. and Asano, T. (1992) Biophys. Chem., in the press.
6 Yamazaki, M., Ohnishi, S. and Ito, T. (1989) Biochemistry 28, 3710–3715.
7 Ito, T., Yamazaki, M. and Ohnishi, S. (1989) Biochemistry 28, 5626–5630.
8 Yamazaki, M. and Ito, T. (1990) Biochemistry 29, 1309–1314.
9 Ito, T., Yamazaki, M. and Ohnishi, S. (1989) Biophys. J. 56, 707–711.
10 Suzuki, A., Yamazaki, M. and Ito, T. (1989) Biochemistry 28, 6513–6518.
11 Yamazaki, M., Ohshika, M. and Ito, T. (1990) Biochim. Biophys. Acta 1063, 175–177.
12 Feigenson, G.W. (1986) Biochemistry 25, 5819–5825.
13 Yamazaki, M., Miyazu, M. and Asano, T. (1992) Biochim. Biophys. Acta 1106, 94–98.
14 Tardieu, A., Luzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733.
15 Ohki, K. (1991) Biophysics (Japan) 31, 285–292.
16 Tanford, C. (1980) The Hydrophobic Effect, John Wiley & Sons, New York.