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THERMAL CHARACTERIZATION OF A POLYETHYLENEGLYCOL (PEG)-DERIVATIVE INDUCED VESICLE FUSION AS REVEALED BY HIGH SENSITIVITY DIFFERENTIAL SCANNING CALORIMETRY

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SUMMARY

Fusion phenomenon of a sonicated large vesicle induced by a derivative of polyethyleneglycol (PEG) was thermodynamically investigated by high sensitivity heating and cooling differential scanning calorimetry (DSC). These studies indicated that the PEG-derivative penetrates into the hydrophobic region of phosphatidylcholine bilayer membrane and leads to a loosely packed structure of the membrane in the liquid crystal state in preference to the gel state. The vesicle incorporating the PEG-derivative induced a fusion with the pure large vesicle, giving rise to an increase in the vesicle size and a decrease in the phase transition enthalpy of the gel-liquid crystal. Based on the calorimetric data, the fusion process was discussed from the viewpoint of the energetics of the PEG-derivative - lipid and the lipid - lipid interactions operating in the lipid membrane of fused vesicles.

INTRODUCTION

As is known, polyethyleneglycol (PEG) is very effective in causing biological membranes to fuse (ref. 1). In attempts to anchor PEG to membranes, PEG-derivative which has a double-hydrocarbon chain (c=12) was synthesized (ref. 2). In this study, the mode of action of the PEG-derivative in promoting cell fusion was investigated on phospholipid-liposomal systems by high sensitivity differential scanning calorimetry.

METHODS

(1) Materials

Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma Chemical Co. and used without further purification. Polyethyleneglycol (PEG)-derivative (shown in Fig. 1) was synthesized by J. Sunamoto and his co-workers.

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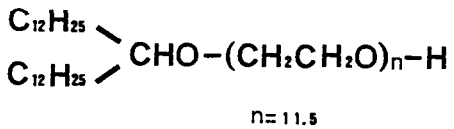

Fig. 1. Molecular formula of PEG-derivative.

(ii) Liposome preparation

Pure vesicle; unilamellar vesicles of DMPC were prepared by first drying the lipid from a chloroform stock solution and resuspending in Tris-buffer solution of PH=7.4. The lipid suspension was then sonicated by a bath sonicator (Branson Ultrasonics Corporation, Model 450) and centrifused at 150,000 xg for 90 min at 30 °C above the phase transition temperature of the lipid. The upper one third of the supernatant was maintained at 30 °C and used for experimental work.

Vesicles containing the PEG-derivative; the stock solution of 50 mM PEG-derivative was prepared by dissolving it in an ethanol/water solvent at a volume ratio of 3/5 and then diluted with the solvent to desired concentrations. Just 10 μl of the PEG-derivative solutions at desired concentrations was added to 2000 μl of the pure vesicle suspension at the lipid concentration of 2mM to prepare the vesicle samples containing the PEG-derivative at desired PEG-derivative [P] / lipid [L] molar ratios. That is, the amount of ethanol is always the same (3.75 μl) for the vesicle samples containing various [P] / [L] molar ratios.

Fused vesicles; the vesicles in the absence and the presence of the PEG-derivative were mixed at desired lipid molar ratios and then annealed by thermal cycling above and below the transition temperature to ensure an equilibrium mixing.

(iii) High sensitivity differential scanning calorimetry

Temperature scanning calorimetric experiments were performed using a Microcal MC-2 differential scanning calorimeter. The calorimeter was interfaced to an IBM PC microcomputer system using an A/D converter board (Data Transition DT-2801) for automatic data collection and analysis. Cooling Scan was achieved with a high precision refrigerated circulating water bath (Haake F3-C) operating under computer control at a desired scanning rate. The lipid concentration in the calorimetric experiments was 2 μmol/ ml with a calorimeter cell volume of 1.2 ml. A scanning rate of 45 °C/h was used for heating scans and -30 °/h for cooling scans. Each sample was repeatedly scanned until the thermogram did not change with time. All the results reported in this paper correspond to thermal equilibrium states of the samples, unless otherwise specified.

RESULTS

(i) Thermal characterization of sonicated pure vesicles

Figure 2 shows the thermotropic behaviors associated with the gel-liquid crystal transition of sonicated pure vesicles of DMPC. As shown in Run I of a cooling scan, the phase transition of the vesicle sonicated at conditions of a output power of 65 w and a time interval of 60 min is relatively broad and is characterized by a transition temperature (t_m) of 19.5 °C, a transition enthalpy (ΔH) of 4.0 kcal/(mole of lipid) and a cooperative unit of 25 lipid molecules. These transition characters are not changed by sonications of intervals longer than 60 min and of powers stronger than 65 w. Furthermore, as far as the vesicle thus obtained is maintained at temperatures above the t_m, the transition profile is not affected for at least four days investigated. As shown in Run II of a heating scan, however, a cooling of the vesicle to temperatures below the t_m causes the appearance of a second peak of a rather sharp shape at around 24.3 °C. This second peak becomes more prominent with a time interval of the cooling procedure, contrary to a gradual diminution of the primary peak at 19.5 °C, as shown in Runs III and IV of heating scans. After about seven days, the second peak becomes the largest, as shown in Run V. The system may be finally composed of a single component. The transition is characterized by t_m of 24.3 °C, ΔH of 4.5 kcal/(mole of lipid) and cooperative unit of ~60 lipid molecules. The sizes of representative two vesicles characterized by each of the primary and second peaks of Fig. 2 were determined by a dynamic light-scattering technique at 31 °C above the t_m, and the mean diameters of these vesicles are ~50 and ~120 nm, respectively. This fact indicates that the cooling procedure below the transition temperature induces a spontaneous fusion of small unilamellar vesicles (SUV's) into large unilamellar vesicles (LUV's) (refs. 3-4). The large unilamellar vesicle thus obtained is stable for at least ten days investigated, and vesicle fusion experiments in the present study were carried out with this large unilamellar vesicle.

(iii) Thermal investigation of interaction of the large unilamellar vesicle with the PEG-derivative.

Figure 3 shows the effect of the PEG-derivative on the thermotropic behavior associated with the gel-liquid crystal transition of the large unilamellar vesicle of DMPC. The addition of the PEG-derivative up to the PEG-derivative [P] / lipid [L] molar ratio of 1/10 causes a progressive increase in the peak area of the transition and also a progressive decrease in the transition temperature as shown in curves 1~4 of Fig. 3. At higher concentration the effect of the PEG-derivative on the t_m and ΔH is essentially the same, but the transition becomes gradually broader and asymmetric, indicating a phase

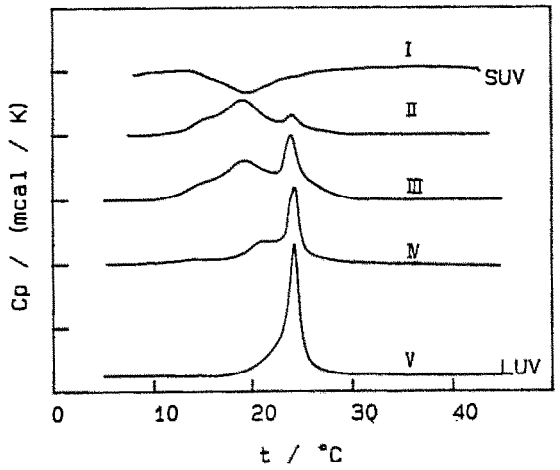

Fig. 2. Thermotropic behaviors of gel-liquid crystal phase transition of sonicated pure vesicles of DMPC. Curve I was obtained in cooling scan and curves II - V were obtained in heating scans.

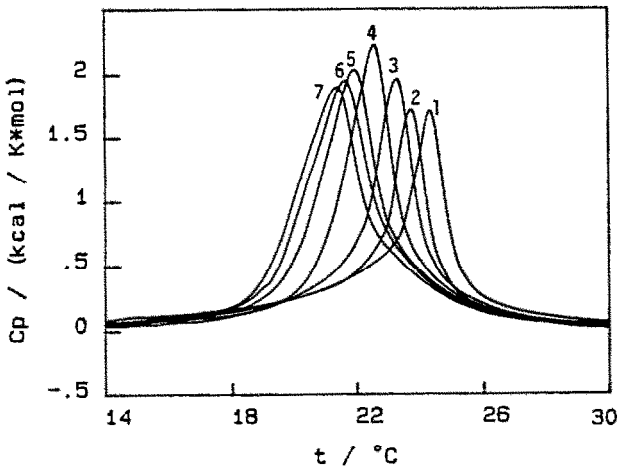

Fig. 3. Thermotropic behaviors of gel-liquid phase transition of DMPC large unilamellar vesicle in the presence of PEG-derivative at [P]/[L] molar ratios of 0 (curve 1), 0.025, 0.05, 0.1, 0.125, 0.15 and 0.20 (curves 2-7).

separation type of phenomenon. Figure 4 shows the dependence of the transition temperature, enthalpy change and cooperative unit on the [P]/[L] molar ratio. These calorimetric data suggest the following results :

(1) At all [P]/[L] molar ratios studied, the PEG-derivative induces a perturbation of the hydrophobic core of the lipid membrane, as evidenced by the decrease in t_m and the increase in ΔH for the transition. That is, the long

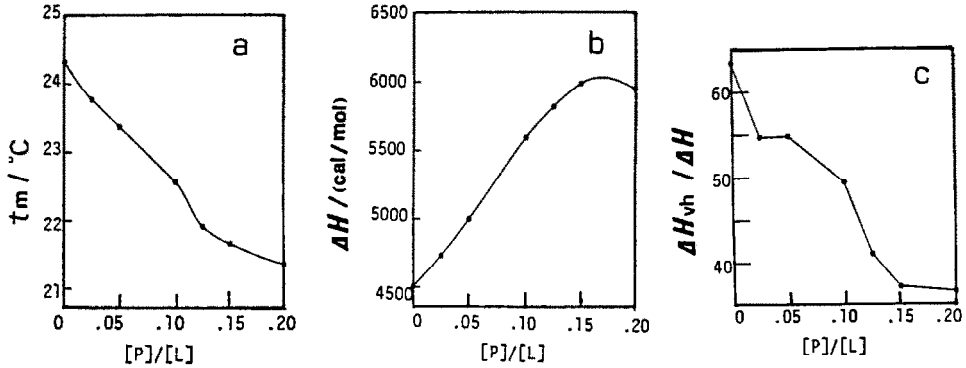

Fig. 4. Effect of PEG-derivative on transition temperature (t_m), transition enthalpy (ΔH) and cooperative unit ($\Delta H_{vh}/\Delta H$) associated with gel-liquid crystal phase transition of DMPC large unilamellar vesicle.

hydrocarbon chain of the PEG-derivative penetrates into the hydrophobic regions of the lipid membrane, giving rise to an increase in their fluidity.

(2) This disordering effect of the PEG-derivative on the lipid membrane is more predominant for the liquid crystal phase and becomes more effective with the increased [P]/[L] molar ratios up to 0.1; This behavior is reflected in the observed increase in ΔH up to this molar ratio.

The measured mean diameters of the vesicles in the presence of the PEG-derivative at the [P]/[L] molar ratios of 0.05 and 0.1 were ~125 and ~135 nm, respectively.

(iii) Thermal characterization of fused vesicles

The vesicle containing the PEG-derivative at the [P]/[L] molar ratio of 0.1 was mixed with the pure vesicle at lipid molar ratios of 2/15 and 6/11, respectively. The thermotropic behaviors of these mixed vesicles thus obtained are shown in curves 3 and 4 of Fig. 5, together with those of the pure vesicle and the vesicle in the presence of the PEG-derivative in curves 1 and 2.

As shown in Fig. 5, the two mixed vesicles show the transition profile of one component. The transition temperatures are located at temperatures between the t_m of the pure vesicle and that of the vesicle in the presence of the PEG-derivative, indicating a dependence of the mixed vesicles on the total [P]/[L] molar ratios. These facts suggest a homogeneous distribution of the PEG-derivative in the lipid membrane of the mixed vesicles. Furthermore, the measured mean diameters of the mixed vesicles at the lipid molar ratios of 2/15 and 6/11 are 151 and 142 nm, respectively, and are larger than those of the vesicles in the absence and presence of the PEG-derivative before a mixing. The increase in the size of the mixed vesicles suggests that :

(1) The vesicle containing the PEG-derivative induces a fusion with the

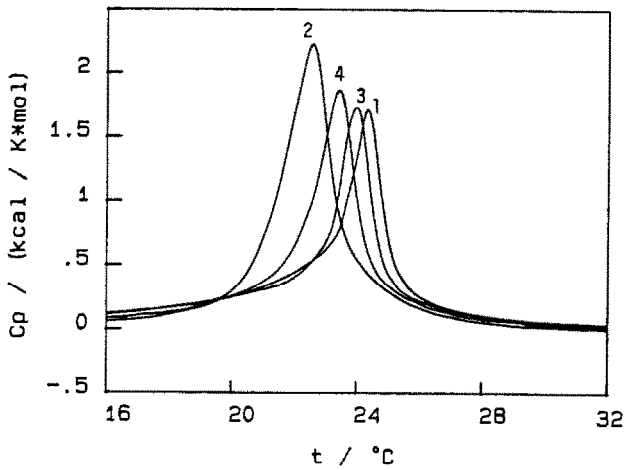

Fig. 5. Thermotropic behaviors of gel-liquid crystal phase transition of mixed vesicles (curves 3 and 4) prepared from pure vesicle (curve 1) and vesicle (curve 2) containing PEG-derivative at [P]/[L] molar ratio of 0.1. Lipid molar ratios of pure vesicle : vesicle in the presence of PEG-derivative in the mixed vesicles are 15:2 and 11:6 for curves 3 and 4, respectively.

pure vesicle which has completed a spontaneous fusion with each other as evidenced by the thermotropic behavior in curve V of Fig. 2.

(2) The homogeneous distribution of the PEG-derivative in the lipid membrane of the mixed vesicles is caused by the vesicle fusion, not by its self-diffusion into the pure vesicle.

DISCUSSION

The PEG-derivative causes an increase in both the transition enthalpy and vesicle size ; the transition enthalpy of 4.5 kcal/mol and the vesicle size of ~120 nm for the pure vesicle are increased up to 5.6 kcal/mol and ~135 nm, respectively, for the vesicle containing the [P]/[L] molar ratio of 0.1. This fact indicates that the PEG-derivative induces a loosely packed structure of the lipid membrane in the liquid crystal state in preference to the gel state. As shown in Fig. 4b, the transition enthalpies of the vesicles in the presence of the PEG-derivative linearly increase with the [P]/[L] ratio up to 0.1. By fitting these experimental data to a linear function by the least squares procedure, the transition enthalpy, ΔH_p, of the vesicle in the presence of the PEG-derivative is given by equation (1) :

$$\Delta H_p = \Delta H_o + 11.0\ [P]/[L] \qquad (1)$$

where ΔH_o is the transition enthalpy of the pure vesicle characterized by the t_m of 24.3 °C, cooperative unit of ~60 lipid molecules and vesicle size of ~120

nm. The second term of this equation corresponds to the amount of change in the transition enthalpy, ΔH_0, due to the PEG-derivative - lipid interaction and depends on the [P]/[L] molar ratio. Therefore, the disordering effect of the PEG-derivative on the lipid membranes in both the gel and liquid crystal phases is reflected in this second term which is, hereafter, called $\Delta H(P\text{-}L)$. In order to investigate the fused vesicle from the thermodynamic viewpoint, the transition enthalpies of fused vesicles at various compositions of the vesicle containing the PEG-derivative at the [P]/[L] ratios of either 0.05 or 0.1 to the pure vesicle were experimentally obtained from the transition peaks represented by curves 3 and 4 of Fig. 5, and were compared with the transition enthalpies, ΔH_p, calculated according to equation (1) using the total [P]/[L] molar ratios of the mixed vesicles ; the experimental transition enthalpy is smaller than the calculated value of the same [P]/[L] ratio and the differences are in the range of 0.6~ 0.8 kcal/(mole of lipid) as shown in Table I.

The actual transition enthalpies of the fused vesicles inconsistent with the calculated values provide an additional support to the idea of nonself-diffusion of the PEG-derivative incorporated by one vesicle into the other pure vesicle. Furthermore, the transition enthalpy of the fused vesicle smaller than the calculated value suggests that the vesicle fusion investigated induces a tightly packed structure of the lipid membrane due to an enhanced lipid-lipid

TABLE I.
Comparison of experimental transition enthalpies (ΔH_f) of fused vesicles at various compositions with their calculated transition enthalpies (ΔH_p).

vesicle sample	ΔH_f/(kcal/mol)	ΔH_p/(kcal/mol)
pure vesicle (1)	—	4.5
vesicle-10 mol% PEG (2)[a]	—	5.6
vesicle-5 mol% PEG (3)[b]	—	5.0
fused vesicle (1+2), 1:1[c]	4.4	5.0
11:6[d]	4.2	4.9
15:2[e]	4.1	4.6
fused vesicle (1+3), 1:1[f]	4.0	4.8
11:6[g]	4.1	4.7
15:2[h]	4.0	4.5

[a] Vesicle containing PEG-derivative at [P]/[L] molar ratio of 0.1.
[b] Vesicle containing PEG-derivative at [P]/[L] molar ratio of 0.05.
[c-e] Fused vesicles prepared from pure vesicle (1) and vesicle (2) containing PEG-derivative at [P]/[L] molar ratio of 0.1; lipid molar ratios of (1)/(2): 1/1, 11/6 and 15/2.
[f-h] Fused vesicles prepared from pure vesicle (1) and vesicle (3) containing PEG-derivative at [P]/[L] molar ratio of 0.05; lipid molar ratios of (1)/(3): 1/1, 11/6 and 15/2.

interaction (ref. 5). Most likely, this ordering effect on the lipid membrane is more effective for the liquid crystal phase than the gel phase. Taking into account the ordering effect on the lipid membrane of fused vesicle, the transition enthalpy, ΔH_f, of the fused vesicle is given by equation (2) :

$$\Delta H_f = \Delta H_p + \Delta H(1\text{-}1) \qquad (2)$$

where $\Delta H(1\text{-}1)$ corresponds to the amount of change in the transition enthalpy, ΔH_p, due to the lipid - lipid interaction and $\Delta H(1\text{-}1) < 0$.
By combining equations (1) and (2), the following equation (3) is obtained :

$$\Delta H_f = \Delta H_o + \Delta H(P\text{-}1) + \Delta H(1\text{-}1) \qquad (3)$$

As a summary of the present thermal investigation of vesicle fusion, the following results will be introduced ; a mixing of the pure vesicle with the vesicle incorporating the PEG-derivative leads to vesicle aggregation and subsequent fusion. At this point, *a homogeneous redistribution of the PEG-derivative molecule* in fused membranes first occurs, and successively followed by *a rearrangement of the lipid molecule* to attain a thermal equilibrium and stable state of the fused vesicle. Based on this feature of the vesicle fusion process, the present study provides equation (3) expressing the transition enthalpy of fused vesicle, which takes into account two predominant enthalpy effects due to the PEG-derivative - lipid and the lipid - lipid interactions operating in the membranes of both the gel and liquid crystal states.

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