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Fluorescence behavior of porphinato Zinc derivative in the molecular assembly of polymerized lipid

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SUMMARY

3,8,13,17-Tetramethyl-7,12-dicarboxy-2,18-bis(octadecyloxycarbonylethyl)porphinato zinc (DCPZn) was incorporated into bilayer membrane of liposomes of 1,2-bis(2,4-octadecadienoyl)-sn-glycero-3-phosphocholine (DODPC). The liposomes were polymerized by initiators like water-insoluble azobisisobutyronitrile (AIBN) or/and water-soluble azobis(amidinopropane) dihydrochloride (AAPD). DCPZn does not disturb the molecular packing of DODPC in the bilayer and after the polymerization, DCPZn is fixed monomolecularly in the network of the crosslinked bilayer membrane, while in the monomeric bilayers the dispersion state of DCPZn depends on temperature and concentration.

INTRODUCTION

Lipid liposomes are typical molecular assemblies which are collecting keen interest in their applications as biomembrane models, drug delivery systems, and so on. Liposome systems however are not stable enough for practical use. The polymerization of liposomes has therefore been vigorously studied to construct stable bilayer structures and the basic physico-chemical properties for these polymerized liposomes have been investigated for the design of actually stable matrix[1-6]. Several unique liposomes, such as corked liposome[7], ghost liposome[8], skeletonized liposome[9], etc., have been prepared through the polymerization. In the previous papers[10-12], we have reported the selective polymerization of diene-type lipids as liposomes and the stability of the polymerized bilayer membranes. For the incorporation of hydrophobic compounds into bilayer membrane, it is very

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important to analyze the effect of polymerization on the distribution and dispersion state of the incorporated materials. In the present study, chemically modified porphyrin derivative is incorporated into monomeric lipid liposomes, and these systems are polymerized by water-soluble or/and water-insoluble radical initiators. The relationship between the fluorescence behavior of porphyrin derivative and the phase transition of lipid bilayer is discussed.

EXPERIMENTAL

Materials

Dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma, 1,2-bis(2,4-octadecadienoyl)-sn-glycero-3-phosphocholine(DODPC) was purchased from Nippon Oil & Fats Co. Ltd. Anthraquinone-2,6-disulfonic acid sodium salt (AQDS) was purchased from Kanto Chem. Co. Ltd. and was recrystallized from alcohol/water mixed solvent. 3,8,13,17-Tetramethyl-7,12-dicarboxy-2,18-bis(octadecyloxycarbonylethyl)porphinato zinc (DCPZn) was synthesized from protoporphyrin-IX sodium salt according to the method which would be reported elsewhere.

Methods

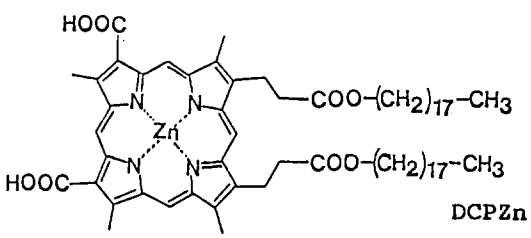
Incorporation of porphyrin into lipid liposomes and their polymerization: Amphiphilic porphinato zinc derivative (DCPZn) was dissolved in chloroform with suitable amount of lipids. The solution was slowly evaporated with a rotary evaporator to form a transparent lipid thin film on the inner wall of flask. Phosphate buffer or distilled water was added and the solution was sonicated (Tomy Seiko UR-200P) for 20 min at 60 w under nitrogen atmosphere. DODPC and porphyrin were similarly dissolved in chloroform which contained azobisisobutyronitrile (AIBN) (5 mol% to DODPC). The liposome suspension was prepared by the same method. For the complete polymerization of DODPC, 5 mol% (to DODPC) of water-soluble azobis(2-amidinopropane) dihydrochloride (AAPD) was then added to the liposome suspension. The solution was bubbled with nitrogen for 15 min and polymerized at 60 ^{o}C for 6 hr under nitrogen atmosphere. The polymerization of DODPC could be confirmed by the disappearance of the UV band at 255 nm attributed to the diene groups[10]. The incorporation of DCPZn into liposomes was confirmed by GPC separation, ultracentrifugation and transmission electronic micrograph (TEM)[13]. GPC elution curve for porphyrin was completely the same as that for lipid liposome. The supernatant of ultracentrifugation of liposome suspension contained neither porphyrin nor lipids. The structure of porphyrin-containing liposomes was

confirmed by TEM to be SUV with average diameter of about 50 nm.

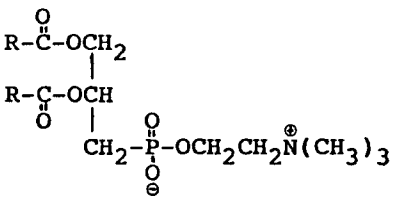
Quenching and temperature dependence of fluorescence intensity of porphyrin: Fluorescence spectra were measured with JASCO FP-770 spectrofluorometer with a temperature-controlled unit. DCPZn was excited at 433 nm. AQDS was dissolved in phosphate buffer of pH 7.0 as 0.1 M stock solution, and was added quantitatively to the liposome suspension to quench the fluorescence of DCPZn. The concentration of DCPZn and the ionic strength of the liposome suspension were kept constant by the addition of phosphate buffer or KCl solutions. Liposome suspensions were heated or cooled in the temperature range between 10 ^{o}C and 70 ^{o}C, and the corresponding fluorescence spectra were recorded. The concentration of porphyrin and the molar ratio of lipid to porphyrin were 1.0 x 10^{-6} M and 300 : 1 respectively except in special mentioned.

RESULTS AND DISCUSSION

Porphyrin DCPZn was incorporated into monomeric DODPC, DPPC or DMPC liposomes with sonication method. Radical polymerization of DODPC liposome with AIBN or/and AAPD was carried out at 60 ^{o}C under nitrogen atmosphere. The presence of porphyrin in liposomes did not show observable interference to the polymerization of lipid liposomes. As in the case of pure liposomes, porphyrin-containing liposomes were polymerized smoothly and reached about 50% conversion by either AIBN or AAPD, but about 90% conversion was obtained when AIBN and AAPD were used simultaneously in 6 hr at 60 ^{o}C because of different location of diene groups of DODPC in 1- and 2-acyl chains[10].



DCPZn



R = $CH_3(CH_2)_{12}$-CH=CH-CH=CH- DODPC

$CH_3(CH_2)_{15}$- DPPC

$CH_3(CH_2)_{13}$- DMPC

The mobilization and the chemical environment of DCPZn in bilayer membranes are affected by the polymerization of the liposomes. Figure 1 is the relationship between the Stern-Volmer constant (Ksv) and the pH of the aqueous suspension. Water-soluble anthraquinone-2,6-disulfonic

acid sodium salt (AQDS) was added to the aqeuous phase to quench the fluorescence from DCPZn in the bilayer membranes. In spite of the quenching mechanism, the larger the Ksv is, the nearer the porphyrin ring to the bilayer/water interface. At pH above 7, due to the ionization of the carboxyl groups of DCPZn, the porphyrin ring of DCPZn was located near the hydrophilic surface of the monomeric lipid bilayer membranes and the fluorescence of DCPZn was quenched remarkably by the water-soluble AQDS as shown in Fig.1. On the other hand, in the pH range of 4 - 7, DCPZn is a neutral molecule and the location of the porphyrin ring should be in the hydrophobic region of the bilayer membrane. The quenching for DCPZn fluorescence showed smaller Ksv. As pH is below 4, the surface property of liposome changed and AQDS tended to be absorbed on the interface of liposome/aqeuous phase. Consenquently, the quenching for DCPZn fluorescence showed larger Ksv. In the bilayer membrane of liposomes, the dissociation of carboxyl groups on porphyrin ring takes place at pH about 7.0. This value is somewhat higher than that in the homogeneous aqueous solution and is in coincident with the titration results for carboxyl group-bearing porphyrins in bilayer membranes of lipid liposomes[14]. After polymerization at pH 6.8 or 9.2, the Ksv for DCPZn showed smaller change with the changing in

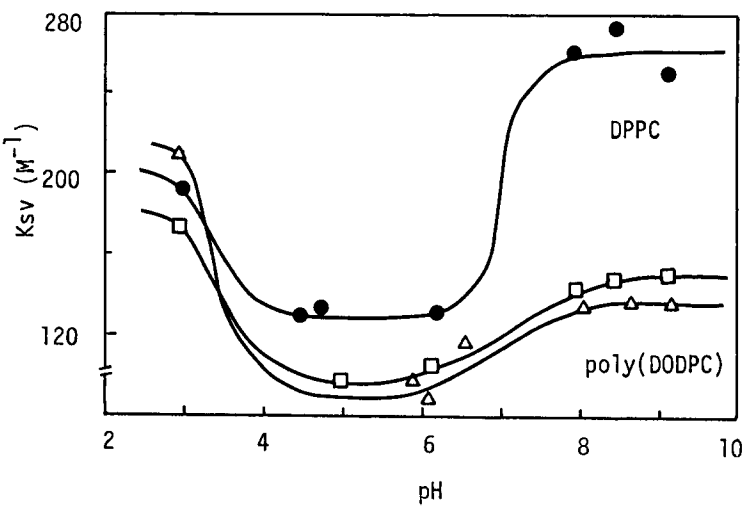


Fig.1 Relationship between solution pH and Stern-Volmer constant (Ksv) quenched by AQDS. DCPZn was incorporated into DPPC liposome (●) or poly(DODPC) liposomes which were polymerized at pH 6.8 (△) or 9.2 (□).

pH in the same range (Fig.1). This is attributed to the fixation of DCPZn in the bilayer membranes. Using AIBN and AAPD as initiators, DODPC liposome is highly crosslinked[1,2]. Porphyrin molecule is expected to be immobilized in the network of poly(lipid) bilayer membrane. The polymerization of porphyrin containing liposomes gives good systems for the investigation of electron transfer reactions through the bilayer/water interface of the liposomes because the location of porphyrin in the bilayer affects greatly the electron transfer reaction yield.

Due to the hydrophobic interaction among porphyrin rings, porphyrin derivatives tend to aggregate or stack, and the fluorescence of porphyrin is consequently quenched[15,16]. In the bilayer membranes, when the molar ratio of lipid to porphyrin was small and the temperature was below the gel-liquid crystalline transition temperature (Tc) of the matrix, the fluorescence of porphyrin was quenched by the concentration quenching effect induced by the stacking as shown in Fig. 2. The porphyrin derivative is partially concentrated or aggregated under this condition.

Figure 3 shows the temperature dependence of fluorescence intensity of DCPZn in monomeric lipid or poly(lipid) liposomes. Phase transition temperature (Tc) determined with DSC for DPPC, DMPC and DODPC are 41 ^{o}C, 23 ^{o}C and 18 ^{o}C, respectively. Poly(DODPC) shows no obvious phase transition. In DPPC system,

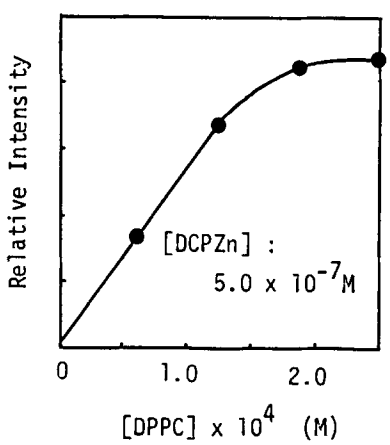


Fig.2 Relationship between fluorescence intensity of DCPZn and concentration of DPPC at 25 ^{o}C. Concentration of DCPZn was 5.0 x 10^{-7} M.

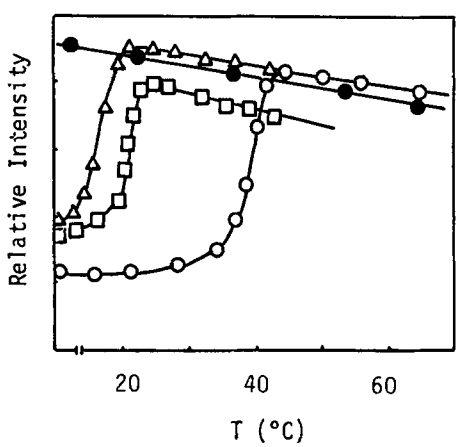


Fig.3 Temperature dependence of fluorescence intensity of DCPZn in various liposomes. Lipids DPPC (○), DMPC (□), DODPC (△), and poly(DODPC) (●) were used.

in spite of the temperature enhanced non-radiation decay of porphyrin excited state, the fluorescence intensity of DCPZn increased slowly, and the transition in fluorescence intensity was found around 41 °C. After this transition point, the fluorescence intensity decreased with increasing temperature. This phenomenon was completely reversible between 10 °C and 70 °C. Because the main phase transition of DPPC lipid takes place at 41 °C, the assembled structure of the matrix affects obviously the dispersion state thus the fluorescence behavior of DCPZn in the bilayer membranes. The fluorescence intensity which reflects the dispersion state of porphyrin can be simply controlled by heating or cooling the liposome suspension at temperature near Tc of DPPC liposome as shown in Fig. 4. In the gel state of the bilayer, porphyrin ring is partially concentrated in the bilayer membrane, consequently, the fluorescence of DCPZn is quenched by the concentration quenching effect. On the other hand, both segmental motion of the acyl chains and lateral diffusion of the lipid molecules become rigorous above the phase transition temperature of DPPC liposome. This rigorous motion perturbs and impacts the neighboring embedded DCPZn molecules. Partially concentrated porphyrin in the gel state of lipid bilayer is dispersed and the concentration quenching for porphyrin fluorescence diminished above the phase transition temperature.

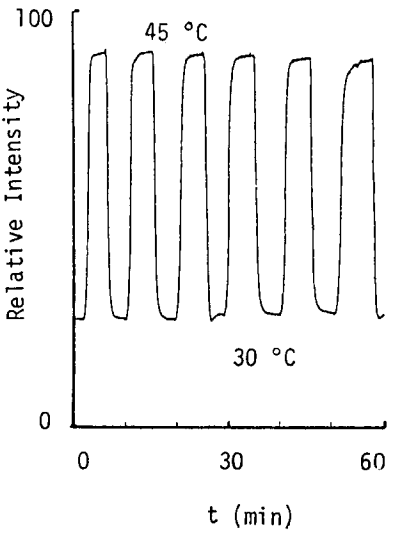


Fig.4 Fluorescence intensity of DCPZn in DPPC liposome with temperature cycling. The liposome was heated or cooled between 30 °C and 45 °C repeatedly.

On the contrary to the case of monomeric lipid liposomes, the fluorescence intensity of DCPZn in poly(DODPC) liposome, which shows no obvious phase transition, decreased monotonously with increasing temperature (Fig.3). This is the same as in organic solvent and is attributed to the temperature enhanced non-radiation decay of the excited state of porphyrin. Because DCPZn was incorporated into monomeric DODPC liposome and then the liposome was polymerized at 60 °C with both AIBN and AAPD, DCPZn was dispersed uniformly and immobilized in the poly(lipid) liposome network. The molecularly dispersed state

of porphyrin ring of DCPZn was therefore kept even at the temperature below the Tc of the corresponding monomer matrix. The temperature dependence of fluorescence intensity for DCPZn in poly(DODPC) liposome proves that this polymeric liposome has no phase transition, and the porphyrin is firmly fixed by the crosslinking of the matrix. As a result, the lateral diffusion of lipid molecules is restricted in the polymeric lipid liposome and the phase separation among bilayer components and DCPZn is suppressed.

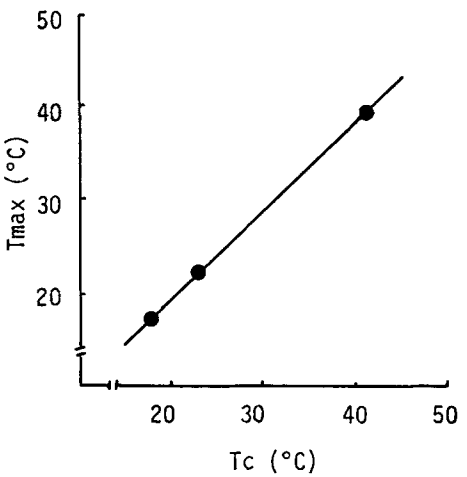


Fig.5 Linear relationship between Tc detected by DSC and Tmax at which the transition in fluorescence intensity of incorporated DCPZn takes place.

The temperature dependence of fluorescence intensity of DCPZn in other monomeric lipid liposomes such as DMPC and DODPC liposomes are also shown in Fig. 3. The curves show that the assembled structure of the matrix have profound effect on the fluorescence intensity of porphyrin in all these case. The temperature (Tmax) for maximum fluorescence transition, determined from the differential curve of the temperature dependence of fluorescence intensity, is plotted against the Tc determined with DSC as shown in Fig. 5. There is a linear relationship between these two set of data. These show us that the fluorescence behavior of porphyrins in the lipid liposomes is certainly related with the phase behavior of the lipid matrices, and can be used as probe to trace the molecular motion and the assembled structure of the bilayer membranes.

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