Polymerization of Unsaturated Phospholipids as Large Unilamellar Liposomes at Low Temperature

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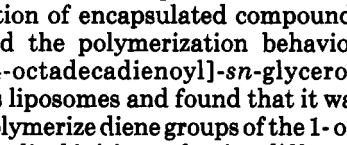
ABSTRACT: Large unilamellar liposomes (LUVs) of unsaturated phospholipids (1,2-bis[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (1) and 1-palmitoyl-2-[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (2)), of which the average diameter was 120 nm, were prepared by extrusion through polycarbonate filters. Polymerization was induced by various redox initiators and γ-irradiation at low temperatures (from 0 to 10 °C). The efficiency of polymerization with a series of redox initiators was in the following order under the same conditions (8 °C, $[\text{initiator}]_0/[1]_0 = 0.05$): Fe^{2+}/*tert*-butyl hydroperoxide > $K_2S_2O_8$/L-cysteine > $K_2S_2O_8/NaHSO_3$, $K_2S_2O_8$/ascorbic acid, $K_2S_2O_8/Na_2S_2O_5$, and $K_2S_2O_8$/glycine > H_2O_2/L-cysteine and H_2O_2/thiourea. The yield of polymerization increased with increasing temperature in the case of $K_2S_2O_8/NaHSO_3$ but decreased in the case of Fe^{2+}/*tert*-butyl hydroperoxide. On the other hand, polymerization under γ-irradiation was proportional to the lipid concentration and the amount of irradiation at 4 °C. The membrane polymerization itself had a small effect on the water-soluble dye dissolved in the inner aqueous phase of liposomes. The degrees of polymerization of lipid polymers were estimated to be 27–68 by gel permeation chromatography after conversion of the polymers of 2 soluble in tetrahydrofuran by methanolization.

Introduction

Liposomes are interesting from the standpoints of model biomembranes and carriers for biologically active substances.[1-3] However, they are not stable against physical and chemical stimuli and are subject to aggregation or precipitation. One of the best methods for making liposomal bilayers more stable is the use of cross-linked bilayers, which are prepared by the polymerization of polymerizable lipids in a bilayer.[4-10] Polymerization of liposomal bilayers can be initiated by various means such as organic initiators,[8,11] UV,[4-6,10] and γ-irradiation.[12,13] The polymerized liposomes are stable in vitro and in vivo.[14,15] However, the degradation of encapsulated compounds such as dyes or enzymes is extensively induced during polymerization by UV irradiation[9] or polymerization at high temperatures. Less attention has been paid to establishing mild conditions, i.e., low temperature, etc., which may cause less degradation of encapsulated compounds.

We studied the polymerization behavior of 1,2-bis[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (1) as liposomes and found that it was possible to selectively polymerize diene groups of the 1- or 2-acyl chain by choosing radical initiators having different solubilities in water.[11,16-20] Since the diene group of the 1-acyl chain was located in the hydrophobic region of the bilayer, it was selectively polymerized with water-insoluble azobis(isobutyronitrile) (AIBN), while that of the 2-acyl chain was polymerized with water-soluble azobis(2-amidinopropane) dihydrochloride (AAPD) because it faced an aqueous phase.



1 : R = $-CH=CHCH=CH(CH_2)_{12}CH_3$

2 : R = $-(CH_2)_{14}CH_3$

However, there are a few studies which have reported on the degrees of polymerization or the structure of the polymers formed.[10b,21-23] Especially in the case of the diene-containing lipid 1, the structure of the polymers was not well understood because the insoluble cross-linked polymers made analyses difficult. Some detailed IR measurements[24] were carried out on the polymerization products of (2*E*,4*E*)-2,4-octadecadienoic acid thin layers, and a few physicochemical measurements[25] indicated only the disappearance of the characteristic signals due to diene groups of 1 but no structure of the product was demonstrated.

In this paper we report the first application of low-temperature redox initiators and γ-irradiation to the membrane polymerization of large unilamellar liposomes (LUVs) of the diene-containing phospholipids 1 and 2 at low temperatures (from 0 to 10 °C). The effect of the polymerization reaction on a water-soluble dye in an inner aqueous phase of the LUVs is studied. Furthermore, the degrees of polymerization and structures of resulting lipid polymers are evaluated by gel permeation chromatography (GPC) and by the use of high-resolution ^1H NMR, respectively.

Experimental Section

Materials. 1,2-Bis[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (1) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) were purchased from Nippon Oil & Fats Co., Ltd. 1-Palmitoyl-2-[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (2) was synthesized according to the method in ref 26. The purity of the lipid was confirmed by thin-layer chromatography (Merck silica gel, 60 plates) with chloroform/methanol/water (65/25/4, by volume). Samples showing a single spot with an R_f value of around 0.4 were used for the experiments. Cholesterol from Wako Pure Chemicals Industry, Ltd., was recrystallized from methanol. Methylene blue purchased from Kanto Chemical Co., Ltd., was recrystallized twice from ethanol. Lipid mixtures were freeze-dried from distilled benzene prior to liposome preparation. Sepharose CL-4B was purchased from Pharmacia Fine Chemicals. Potassium persulfate ($K_2S_2O_8$), L-cysteine, sodium hydrogen sulfite ($NaHSO_3$), ascorbic acid, sodium pyrosulfite ($Na_2S_2O_5$), glycine, hydrogen peroxide (H_2O_2), L-cysteine, thiourea, iron(II) chloride ($FeCl_2$), and *tert*-butyl hydroperoxide were purchased from Kanto. $K_2S_2O_8$ was purified by recrystallization twice from distilled water, and the others

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were used without further purification. Triton X-100 was purchased from Tokyo Kasei Co., Ltd., and was used without further purification.

Preparation of Liposomes. Large unilamellar liposomes of 1 or 2 were prepared by the extrusion method using an extruder (Lipex Biomembrane Inc.). A freeze-dried powder (0.5 g) of 1 or 2 was suspended in distilled water (10 mL), and the mixture was vortexed with 2 mL of glass beads (diameter 2–3 mm) under an argon atmosphere above the phase transition temperature (T_c) of 1 (18 °C) or 2 (25 °C) for 15 min. The suspension was extruded through polycarbonate filters (pore size 2.0, 1.0, 0.6, 0.4, 0.2, and then 0.1 μm) above T_c.

An aqueous solution of methylene blue (0.3 mmol dm^{-3}) was encapsulated with the lipid mixture of 1 and cholesterol or DSPC and cholesterol (molar ratio 1/1) to prevent leakage of the dye. Free methylene blue was removed by gel permeation chromatography on Sepharose CL-4B at 5 °C.

The average diameters of the liposomes were determined by quasi-elastic light scattering measurements (Coulter N4, Coulter Electronics Co.).

Polymerization Methods. The polymerization of LUVs by γ-irradiation was carried out by using ^{60}Co (3000 Ci). The lipid suspensions (lipid concentration 12.8–89.6 mmol dm^{-3}) were sealed in glass tubes after bubbling with argon through the solution. The glass tubes were placed in a Dewar at 4 and 25 °C. The reaction table rotated and the Dewar revolved around the ^{60}Co to give an averaged irradiation intensity.

The polymerization of LUVs by the addition of the redox initiators was carried out under an argon atmosphere at a temperature of 8 °C. The reaction was started by the simultaneous addition of prescribed amounts of 5 wt % aqueous solution of each component of a redox initiator. The polymerization was followed by measuring the decrease of UV absorption intensity at 255 nm due to the diene chromophores of 1 or 2.

Phase Transition Measurements. The temperature dependence of the absorption maximum (λ_{max}) of the diene groups of the liposome suspensions was measured in a quartz cell (path length 10 mm) by a UV spectrophotometer (Shimadzu MPS-2000) with increasing or decreasing temperature by the rate of 1 °C min^{-1}.

Analyses of Lipid Polymers. The degree of polymerization was estimated by analyzing the lipid polymers derived from the polymerization of the LUVs of 2 having only one polymerizable group in the molecule. The lyophilized membranes were methanolized in 20% HCl/absolute methanol in an oil bath (100 °C) for 2 days.[10b] After the solvent was removed under reduced pressure, the residue was dissolved in chloroform, washed with water, and then dried. Anal. Calcd for $C_{19}H_{34}O_2$: C, 77.50; H, 11.64. Found: C, 77.20; H, 11.82. The average molecular weights of the methanolized polymers were determined by high-performance liquid chromatography with AD-803/AD-804/AD80M/AD-802 GPC columns (Shouwa Denkou Co.), using tetrahydrofuran as solvent. The column was calibrated with polystyrene standards. The 400-MHz ^{1}H NMR spectra of the methanolized polymers were measured in $CDCl_3$ (TMS as internal standard) on a JEOL GSX-400 NMR spectrometer.

Results and Discussion

1,2-Bis[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (1) and 1-palmitoyl-2-[(2*E*,4*E*)-2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (2) were dispersed in distilled water, and large unilamellar liposomes were prepared by the extrusion method. The sizes and shapes of the liposomes were determined by a quasi-elastic light scattering and transmission electron microscopic (TEM) measurements. They supported the formation of unilamellar liposomes with an average diameter of 120 nm.

Polymerization by Redox Initiators. The following redox initiators form various radicals at low temperatures[27–31] and initiate the polymerization of the LUVs of 1.

$$Fe^{2+} + (CH_3)_3COOH \rightarrow Fe^{3+} + (CH_3)_3CO^* + OH^- \quad (1)$$

$$HSCH_2CH(NH_2)COOH + S_2O_8^{2-} \rightarrow {}^*SCH_2CH(NH_2)COOH + SO_4^{-*} + HSO_4^- \quad (2)$$

$$HSO_3^- + S_2O_8^{2-} \rightarrow HSO_3^* + SO_4^{-*} + SO_4^{2-} \quad (3)$$

$$\underline{OCOC(OH):C(OH)}CHCH(OH)CH_2OH + S_2O_8^{2-} \rightarrow \underline{[OCOCO:COC}HCH(OH)CH_2OH]^{-*} + SO_4^{-*} + H_2SO_4 \quad (4)$$

$$S_2O_5^{2-} + H_2O \rightarrow 2HSO_3^-$$

$$HSO_3^- + S_2O_8^{2-} \rightarrow HSO_3^* + SO_4^{-*} + SO_4^{2-} \quad (5)$$

$$HSCH_2CH(NH_2)COOH + H_2O_2 \rightarrow {}^*SCH_2CH(NH_2)COOH + {}^*OH + H_2O \quad (6)$$

$$H_2NC(S)NH_2 + H_2O_2 \rightarrow HN{=}C(-S^*)NH_2 + {}^*OH + H_2O \quad (7)$$

The polymerization behavior of 1 as LUVs initiated by the various redox initiator compounds at 8 °C is shown in Figure 1. The initial molar ratio of a redox initiator to 1 was kept at 0.05. Polymerization was effectively induced in the case of Fe^{2+}/*tert*-butyl hydroperoxide and $K_2S_2O_8$/L-cysteine. This excellent efficiency was due to the very low activation energy of the redox reactions (for example, E_a = 9.8 kcal/mol for the former[27]). Polymerization proceeded efficiently in the following order of radical initiators under the same conditions: Fe^{2+}/*tert*-butyl hydroperoxide > $K_2S_2O_8$/L-cysteine > $K_2S_2O_8$/$NaHSO_3$, $K_2S_2O_8$/ascorbic acid, $K_2S_2O_8$/$Na_2S_2O_5$, and $K_2S_2O_8$/glycine > H_2O_2/L-cysteine, while slow reaction was observed for $K_2S_2O_8$/thiourea and H_2O_2/thiourea. No polymerization was confirmed after the addition of one component of a redox initiator mixture at such a low temperature (8 °C). When oxidant and reductant components coexisted, polymerization occurred smoothly.

In the case of $K_2S_2O_8$/$NaHSO_3$, the activation energy for decomposition is 12.5 kcal/mol and the radicals are formed through reactions 3 and 8; the initiator radicals formed become inactive by coupling via reactions 9 and 10.[29] It has been found for the polymerization of methyl

$$SO_4^{-*} + H_2O \rightarrow HSO_4^- + {}^*OH \quad (8)$$

$${}^*OH + HSO_3^* \rightarrow H_2SO_4 \quad (9)$$

$${}^*OH + HSO_3^- \rightarrow {}^*HSO_3^- + OH^- \quad (10)$$

methacrylate by redox initiators in water that the end groups of the polymers were sulfoxy groups rather than hydroxy groups under conditions where the concentration of the initiator was higher than 0.5 mmol dm^{-3}.[29] Therefore, under the present initiator concentration (0.64 mmol dm^{-3}), the polymerization of 1 is believed to be initiated by HSO_3^* and not *OH. Figure 2 shows that the polymerization yield increased and saturated at 53% after multiple addition of the 5 mol % redox initiators at 8 °C. At 8 °C the bilayer is gel state because the phase transition

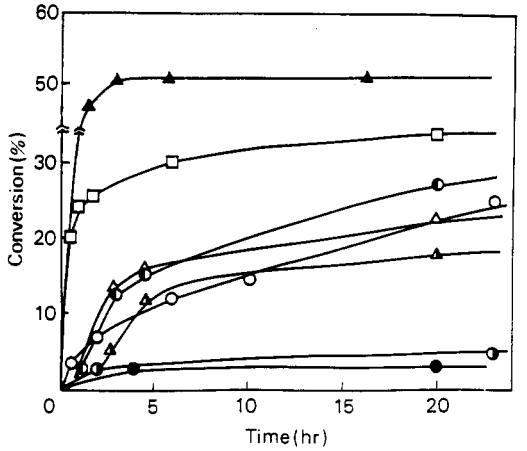

Figure 1. Polymerization of the 1 LUVs by various redox initiators at 8 °C: $K_2S_2O_8$/L-cysteine (□), $NaHSO_3$ (○), ascorbic acid (△), $Na_2S_2O_5$ (▲), glycine (◑), H_2O_2/L-cysteine (◐), thiourea (●), Fe^{2+}/*t*-BuOOH (▲). [1] = 12.8 mmol dm^{-3}, [initiator]/[1] = 0.05, [reductant]/[oxidant] = 1.

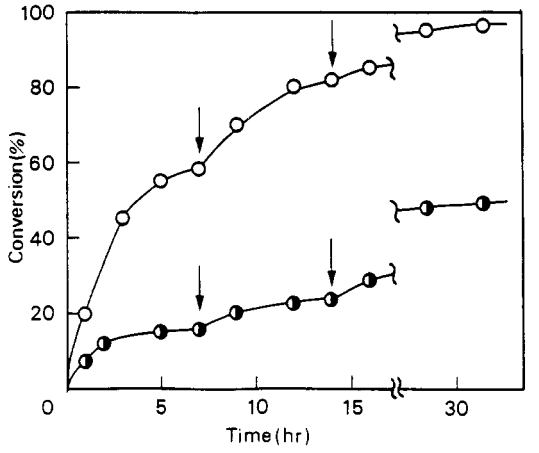

Figure 2. Polymerization of 1 LUVs by continuous addition of the redox initiator at 8 °C (◑) and 30 °C (○). [1] = 12.8 mmol dm^{-3}, $[K_2S_2O_8]/[1] = 0.05$, $[NaHSO_3]/[K_2S_2O_8] = 5$.

temperature (T_c) is 18 °C. Less permeation of such ionic species is expected to occur through the hydrophobic bilayer membrane. Therefore, the saturation of polymerization suggested the polymerization of only the outward-facing lipids of vesicles.[16-20] In this case, the diene groups of 1- and 2-acyl chains were polymerized nonselectively.[32] At 30 °C ($>T_c$), the polymerization yield was much higher than at 8 °C and complete polymerization was observed. This indicated that at temperature above T_c the initiator compounds or radicals can penetrate the bilayer membrane and initiate the polymerization of both inner- and outward-facing lipids.

In the case of Fe^{2+}/*tert*-butyl hydroperoxide, the efficiency of polymerization at 8 °C was much larger than with other redox initiators because of its low activation energy. Polymerization was initiated by $(CH_3)_3CO^*$. The yield increased and saturated at 55% under the same conditions as for the $K_2S_2O_8/NaHSO_3$ system. In this case, as opposed to the $K_2S_2O_8/NaHSO_3$ system, the polymerization yield decreased with increasing temperature from 8 to 30 °C (Figure 3). This phenomenon may be explained by the so-called "dead-end polymerization".[33] Since the decomposition of the redox initiator (Fe^{2+}/*tert*-butyl hydroperoxide) is more rapid at higher temperature, the initiator radicals formed explosively at the initial stage of the polymerization and reacted with each other, resulting in the ineffective initiation of polymerization.

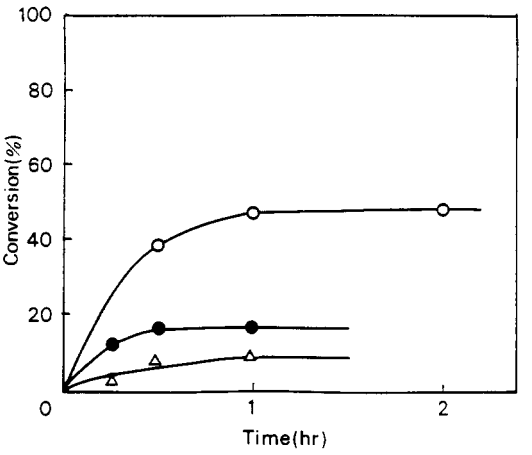

Figure 3. Effect of temperature on the polymerization of 1 LUVs by the redox initiator. [1] = 12.8 mmol dm^{-3}, $[Fe^{2+}]/[t\text{-BuOOH}] = 1$, $[Fe^{2+}]/[1] = 0.05$. Polymerization temperature (°C): 8 (○), 20 (●), 30 (△).

Polymerization by γ-Irradiation. Polymerization of 1 was carried out by γ-irradiation. The polymerization yield saturated at 80% (0.73 Mrad, 4 °C), and no selective polymerization was confirmed. Figure 4 shows the relationship between lipid concentration and polymerization rate. The polymerization rate is proportional to the 1.2th power of the 1 concentration. This indicates that the polymerization of liposomes is initiated by OH radicals generated from decomposition of water, not direct excitation of monomer lipids.

On the other hand, polymerization rate is proportional to $I^{0.92}$ (as shown in Figure 5). In general, polymerization rate is proportional to $I^{0.5}$ because termination occurs by recombination or disproportionation between two growing polymer chains. Therefore, it is suggested that unimolecular termination occurs in the case of radical polymerization in the bilayer membrane.

The following reaction schemes were, therefore, suggested

$$H_2O \xrightarrow{k_d} OH^* + H^* \qquad R_d = d[OH^*]/dt = k_d f[H_2O]I \quad (11)$$

$$OH^* + M \xrightarrow{k_i} M^* \quad (12)$$

$$M_n^* + M \xrightarrow{k_p} M_{n+1}^* \quad (13)$$

$$M_n^* \xrightarrow{k_t} P_n \quad (14)$$

where f, M, and P_n mean initiation efficiency, a growing polymer chain, and a dead polymer chain, respectively. H* does not initiate the polymerization of liposomes because of its low reactivity. If the polymer chains are long and the effective initiating radicals are distributed uniformly, polymerization rate (R_p) would be described as eq 15, where $k = k_p k_d f[H_2O]/k_t$. This shows that R_p is proportional to both [M] and I, satisfying our experimental results.

$$R_p = -d[M]/dt = k[M]I \quad (15)$$

Effect of Polymerization on Dyes in the Inner Aqueous Phase. The effect of membrane polymerization on solutes dissolved in the inner aqueous phase of the LUVs of 1 was then elucidated. Methylene blue was used as a probe because this dye was found to be decolarized

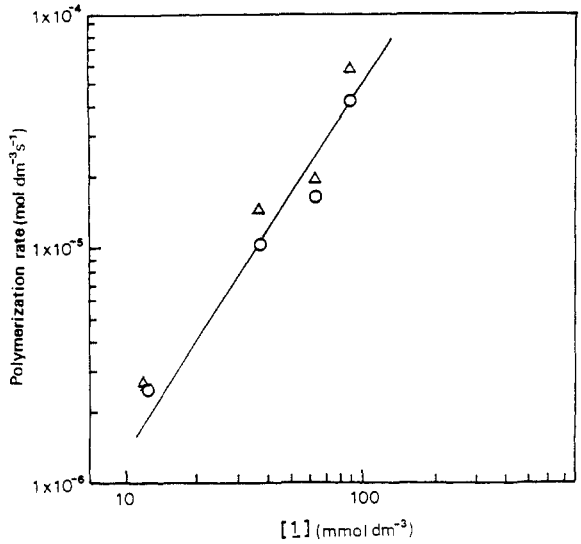

Figure 4. Dependence of polymerization rate on the monomer 1 concentration in the γ-irradiation. Total dose 3.65×10^{-2} Mrad, dose rate 1.53×10^{-1} Mrad h^{-1}. Polymerization temperature (°C): 25 (△), 4 (○).

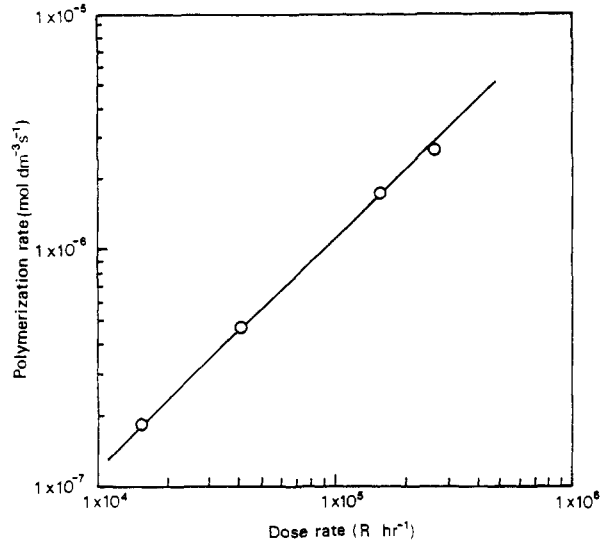

Figure 5. Dependence of polymerization rate on the dose rate of γ-irradiation. Polymerization temperature 4 °C, [1] = 12.8 mmol dm^{-3}, total dose 9.20×10^{-2} Mrad.

by various radicals including polymer growing ends.[32,34] Since the dye leaked very slowly through the liposomal bilayer of 1 or DSPC under the experimental conditions (8 °C, 5% leakage after 24 h), the lipid mixture of 1 and cholesterol or DSPC and cholesterol was used, where no leakage was found. The decolorization of the dye in LUVs during the polymerization by $K_2S_2O_8/NaHSO_3$ or γ-irradiation was followed by measuring the absorbance at 664 nm due to the dye. The polymerization behavior and the decolorization of methylene blue were determined simultaneously on the LUVs having methylene blue in its inner aqueous phase. The polymerization with $K_2S_2O_8/NaHSO_3$ or γ-irradiation proceeded with the same rate as in the absence of methylene blue, indicating no retardation of membrane polymerization by this dye.

Membrane polymerization at 8 °C with $K_2S_2O_8/NaHSO_3$ had only a small effect on the dye in the inner aqueous phase. However, at 30 °C the dye was decolorized rapidly during polymerization. This shows that the radicals or initiator compounds could penetrate the bilayer and decompose the dye in the inner aqueous phase. γ-Irradiation (up to 0.73Mrad) was applied to the LUVs

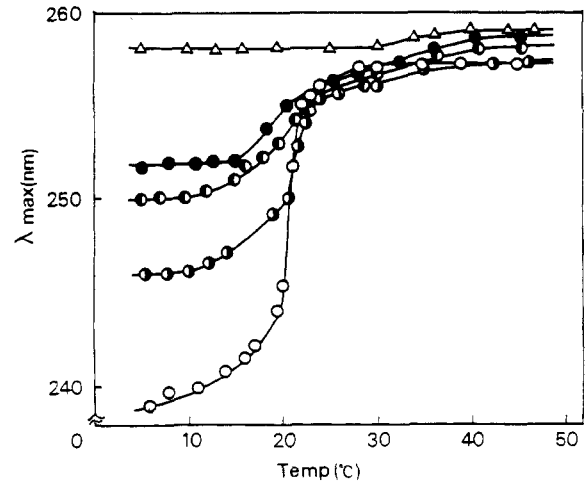

Figure 6. Temperature dependence of the absorption maximum of polymerized 1 LUVs prepared by γ-irradiation with various polymerization conversion: 0% (○), 7% (◑), 24% (◐), 50% (●), 74% (▲). Diameter 111 ± 43 nm.

having the dye at 4 °C. Encapsulated methylene blue was decolorized with the amount of irradiation, and the polymerization proceeded with the same rate independently of the presence of the dye. The rate of decolorization of the dye in the LUVs of 1 and cholesterol was the same as that in the LUVs composed of the nonpolymerizable phospholipid (DSPC) and cholesterol. This indicates that the propagation reaction of 1 in the bilayer does not affect the decolorization reaction and that the decolorization is caused by OH radicals formed within the inner aqueous phase of liposomes.

Temperature Dependence of the Absorption Maximum of Polymerized 1 Liposomes. Figure 6 shows the temperature dependence of the absorption maximum (λ_{max}) of diene groups in polymeric liposomes obtained by γ-irradiation. The unreacted diene groups were employed to analyze the packing state of the acyl chains in the polymeric liposomes.[20] As shown in the figure, the phase transition temperature is almost independent of the polymerization yield up to 50%, while the absorption maximum below T_c depends on it. The former indicates that the phase transition mainly reflects the aggregates of the residual and nonpolymerized diene moieties. The latter corresponds to the averaged state of molecular packing of polymers and monomers. The polymers have an effect on the packing and orientation of diene groups or acyl chains around lipid polymers. No clear transition was observed for polymerized liposomes with at high polymerization yield (74%).

Stability of Polymerized Liposomes. The polymerized 1 liposomes prepared at 8 °C by the 5 mol % redox initiator ($K_2S_2O_8/NaHSO_3$ or Fe^{2+}/*tert*-butyl hydroperoxide, polymerization yield 50%) were stable against a high concentration (12 mmol dm^{-3}) of Triton X-100, while 4 mmol dm^{-3} of the surfactant completely decomposed nonpolymerized liposomes (Figure 7). The average particle diameter of the polymerized liposomes was not changed even after repeated freeze–thaw treatment of the aqueous dispersion, while nonpolymerized liposomes were fused together and particle diameters were changed (data not shown). The stability was almost the same as that of the polymerized liposomes prepared by polymerization initiated by 5 mol % azobis(2-amidinopropane) dihydrochloride at 60 °C (polymerization yield 50%, selective polymerization of the 2-acyl chains of both outward- and inner-facing lipids). This result showed that the stability of the liposomes was sufficiently maintained by the cross-

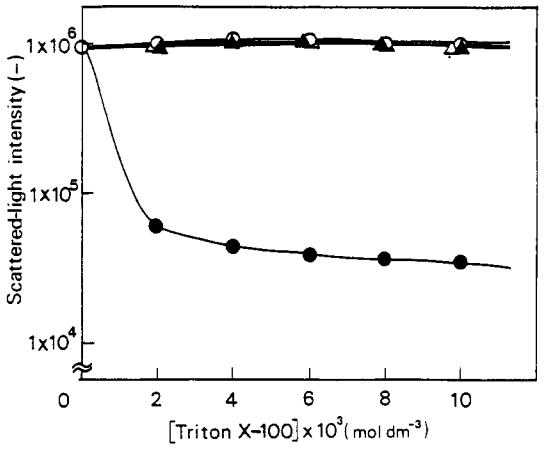

Figure 7. Scattered-light intensity change for polymerized 1 LUVs by the addition of Triton X-100 at 20 °C: nonpolymerized LUVs (●); polymerized LUVs prepared by $NaHSO_3/K_2S_2O_8$ (○), Fe^{2+}/t-BuOOH (▲), and γ-irradiation (△).

Table I
Number- and Weight-Average Molecular Weights ($\bar{M}_n$ and $\bar{M}_w$) and Number- and Weight-Average Degrees of Polymerization ($\overline{DP}_n$ and $\overline{DP}_w$) of Methanolized Polymers Derived from the Large Unilamellar Liposomes of 2 by Various Polymerization Methods

method	temp (°C)	$\bar{M}_n \times 10^{-4}$ ($\overline{DP}_n$)	$\bar{M}_w \times 10^{-4}$ ($\overline{DP}_w$)	$\bar{M}_w/\bar{M}_n$
$NaHSO_3/K_2S_2O_8$	8	0.78 (27)	1.16 (39)	1.49
Fe^{2+}/t-BuOOH	8	0.83 (28)	1.27 (43)	1.53
γ-irradiation	4	1.97 (68)	6.32 (218)	3.20
AAPD	8[a]	0.98 (33)	1.68 (57)	1.71
	20[a]	1.27 (43)	2.99 (102)	2.35
UV	8	0.54 (18)	0.69 (23)	1.20
$NaHSO_3/K_2S_2O_8$	35	1.33 (45)	7.22 (246)	5.43
AAPD	35[a]	1.87 (63)	3.42 (116)	1.82
	60[b]	1.57 (53)	4.54 (154)	2.88

[a] From ref 19. AAPD was decomposed by visible light. [b] From ref 17. AAPD was decomposed by heating (60 °C).

linking of only outward-facing lipids of the bilayer.

Analysis of Lipid Polymers. The lyophilized liposomes of 1 polymerized by redox initiation or γ-irradiation were insoluble in any organic solvent. This result shows that the lipid polymers were cross-linked. Using the liposomes of 2 which has only one polymerizable groups in molecule, polymerization was performed under the same conditions as for 1. Methanolized polymers of 2 were soluble in tetrahydrofuran. The weight- and number-average degrees of polymerization of the polymers are summarized in Table I together with those measured on the lipid polymers prepared by the different polymerization methods. Below T_c the polymerization by γ-irradiation gave polymers with higher molecular weight than did $K_2S_2O_8/NaHSO_3$, $Fe^{2+}/tert$-butyl hydroperoxide, and other systems. Polymerization above T_c gave polymers with higher molecular weight having broader distribution.[23] The propagation may be enhanced by an increase of the thermal movement of the lipids in the bilayer, inducing the diene groups or radicals to take suitable conformation for polymerization.

The structure of the lipid polymers, especially that of the diene-containing lipid 1, has not been well understood because of insoluble cross-linked polymer formed. In the case of amphiphilic dienes in LB films, the mechanism of photopolymerization has been studied by IR, UV, and NMR spectroscopy.[24,35] The diene moiety may undergo the variety of reactions including polymerization. The

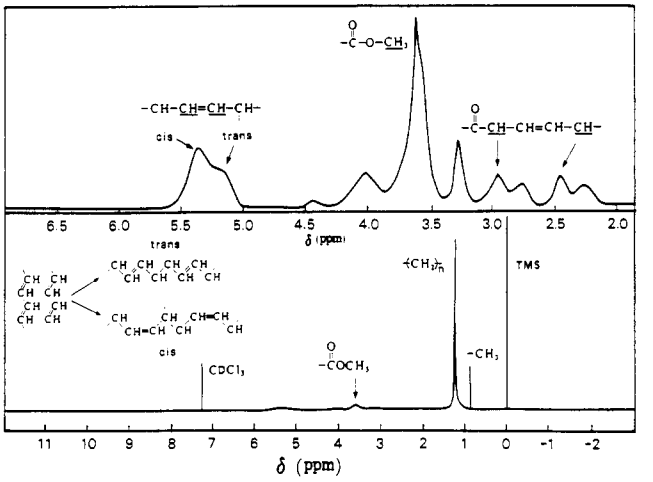

Figure 8. 400-MHz ^1H NMR spectrum of polymers obtained by methanolization. Polymerized LUVs of 2 prepared by the $NaHSO_3/K_2S_2O_8$ initiator ($\overline{DP}_n$ = 27). Solvent $CDCl_3$, internal standard TMS.

Table II
Selective Polymerization of Acyl Chains of 1 as Unilamellar Liposomes by Various Polymerization Methods

method	polymerization moiety: $T < T_c$	polymerization moiety: $T > T_c$
AIBN	no effective polym	1-acyl chain of inner and outer layers
AAPD	2-acyl chain of outer layer[a]	2-acyl chain of inner and outer layers
$NaHSO_3/K_2S_2O_8$	1,2-acyl chains of outer layers	1,2-acyl chains of inner and outer layers
Fe^{2+}/t-BuOOH		no effective polym
UV	1,2-acyl chains of inner and outer layer	
γ-irradiation		

[a] From ref 19. AAPD was decomposed by visible light.

reaction could take place in position 1,2, in position 3,4, or in position 1,4.

As described above, polymers with an average degree of polymerization ranging from 27 to 68 were obtained in the polymerization of 2 as liposomes by redox initiators or by γ-irradiation. The structures of the polymers formed were studied as their methanolized derivatives by high-resolution NMR. The 400-MHz ^1H NMR spectrum of the polymers obtained by methanolization of the 2 polymers prepared by the use of $K_2S_2O_8/NaHSO_3$ is shown in Figure 8. The spectrum supports the structure of the methanolized polymers, and in the region from 5.0 to 5.5 ppm the peaks attributed to the residue formed by 1,4-addition reaction are observed with a small amount of unknown peaks. The spectrum also shows that the isolated double bond (2,3-position) is a mixture of cis and trans forms. Thus, it can be concluded that the polymerization of diene lipid 2 proceeded mainly through 1,4-addition.

Selective Polymerization of Acyl Chains of 1 Liposomes. Table II summarizes the conclusions of our study on the selective polymerization of acyl chains of 1 as unilamellar liposomes by various polymerization methods, obtained by the present work and our previous reports.[16-19] The authors were able to control the polymerization of the acyl chains of 1 LUVs by selecting the polymerization methods and conditions. In the present study, the new polymerization method which induces the polymerization of the diene groups at 1- and 2-acyl chains of the outward-facing phospholipid 1 as LUVs was found by using redox initiators at temperature below the T_c, without affecting the inner solute. γ-Irradiation was very effective even at

low temperature, and the polymerization occurred nonselectively to a high yield.

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