(29) The solvent polarities of the head group region and the inner part of the vesicles, as measured by ANS and NPN, respectively, do not lie on the same polarity scale (Figure 7). The reason for this phenomenon is not clear yet. It could be that the fluorescence behavior of ANS is somehow influenced by the positive and negative charges of the quaternary ammonium functions and the Br^- ions. In separate experiments, however, we found that adding benzyltriethylammonium bromide to solutions of ANS in dioxane–water mixtures did not change the position of the fluorescence maximum.
(30) (a) Forster, Th.; Rokos, K. *Chem. Phys. Lett.* **1967**, *1*, 279–280. (b) Robinson, G. W.; Robbins, R. J.; Fleming, G. R.; Morris, J. M.; Knight, A. E. W.; Morrison, R. J. S. *J. Am. Chem. Soc.* **1978**, *100*, 7145–7150.
(31) Roks, M. F. M., unpublished results.
(32) Nagamura, T.; Mihara, S.; Okahata, Y.; Kunitake, T.; Matsuo, T. *Ber. Bunsenges. Phys. Chem.* **1978**, *82*, 1093–1098.
(33) Galla, H. J.; Sackman, E. *Ber. Bunsenges. Phys. Chem.* **1974**, *78*, 949–953.
(34) Goedheer, J. C. *Photobiochem. Photobiophys.* **1984**, *7*, 91–101.
(35) Ververgaert, P. H. J. Th.; Elbers, P. F.; Luitingh, A. J.; Van de Berg, N. J. *Cytobiology* **1972**, *6*, 86–96.
(36) Schore, N. E.; Turro, N. J. *J. Am. Chem. Soc.* **1975**, *97*, 2488–2496.
(37) Westphal, O.; Jerchel, D. *Chem. Ber.* **1940**, *73*, 1002–1011.
(38) Rothe, M.; Kunitz, F. W. *Justus Liebigs Ann. Chem.* **1957**, *609*, 88–102.
(39) For a discussion, see: Bolikal, D.; Regen, S. L. *Macromolecules* **1984**, *17*, 1287–1289.

Polymerization of Liposomes Composed of Diene-Containing Lipids by UV and Radical Initiators: Evidence for the Different Chemical Environment of Diene Groups on 1- and 2-Acyl Chains

Hiroyuki Ohno, Yoshitaka Ogata, and Eishun Tsuchida*

Department of Polymer Chemistry, Waseda University, Tokyo 160, Japan.
Received March 7, 1986

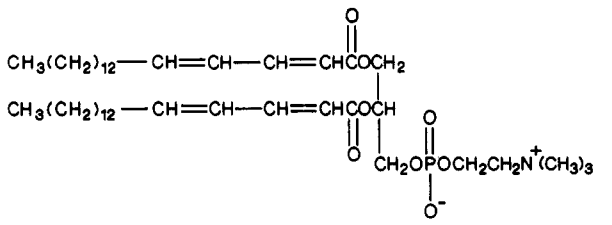
ABSTRACT: 1,2-dioctadecadienoyl-3-phosphatidylcholine (DODPC), a polymerizable lipid that contains one diene group per acyl chain, was polymerized as liposomes. Polymerization was done by UV irradiation or by the addition of a radical initiator. Water-insoluble azobis(isobutyronitrile) (AIBN) and/or water-soluble azobis(2-amidinopropane) dihydrochloride (AAPD) were examined. The UV irradiation for 3 h provided a complete polymerization of DODPC liposomes. Contrary to this, polymerization by the addition of radical initiator (AIBN or AAPD) gave 50–60% polymerization conversion at 60 °C. Complete polymerization was performed by the use of AIBN and AAPD simultaneously. The polymerization conversion was also improved by the addition of AAPD to liposomes that were preliminarily polymerized by AIBN. The DODPC liposomes polymerized by both AIBN and AAPD provided the most stable polymerized liposomes. The liposomes polymerized by both AIBN and AAPD showed the least carboxyfluorescein leakage from the inner aqueous phase. The obtained results strongly suggested that the diene groups in 2-acyl chains should face the aqueous phase, and therefore polymerization was initiated by a water-soluble radical initiator (AAPD) but not by AIBN.

Introduction

Phospholipid bilayer liposomes are gathering keen interest as microcapsules for carriers of drugs or functional particles as well as models for biomembranes.[1,2] These liposomes are generally not very stable and cause aggregation and fusion. To stabilize these assembled structures polymerizable lipids were synthesized as a major component in constructing the membrane. The physicochemical properties of the polymerized liposomes have been collected vigorously in this decade.[1-12] Several unique liposomes were also prepared by polymerization, such as corked liposomes,[13] ghost liposomes,[14] skeletonized liposomes,[15] etc. Most of the polymerizations have been performed by UV irradiation because of its convenience. Although polymerization is one of the most potent techniques in principle, the actual polymerized systems thus prepared have several problems such as distortion of the assembled structure and formation of clefts or cracks in the membranes that were induced by polymerization.[16,17] In this paper, diene-type monomeric lipids were polymerized as liposomes by UV irradiation or by radical initiators to compare the characteristics of the obtained polymeric liposomes.

Experimental Section

Materials. 1,2-di(2,4-octadecadienoyl)-3-phosphatidylcholine (DODPC) was purchased form Nippon Oil & Fats Co. Ltd. This was characterized by thin-layer chromatography (Merck, silica gel plates) with chloroform/methanol/water (65/35/5, by vol) as solvent before use.[17] DODPC showed a single spot with an R_f value of 0.3, similar to that of 1,2-distearoyl-3-phosphatidylcholine (special grade, Sigma).



DODPC

Azobis(isobutyronitrile) (AIBN) and azobis(2-amidinopropane) dihydrochloride (AAPD) were purchased from Tokyo Kasei Co. Ltd. AIBN and AAPD were purified twice by recrystallization from methanol and water, respectively.

Triton X-100, polyethylene glycol mono-*p*-octylphenyl ether ($HO(CH_2CH_2O)_{10}C_6H_4C_8H_{17}$), was purchased from Tokyo Kasei Co. Ltd. and was used without further purification. Fluorescent grade 5(6)-carboxyfluorescein (CF) was purchased from Kodak Co. Ltd.

Methods. A total of 0.200 g of lipids was dissolved in dehydrated chloroform and was slowly evaporated in a rotated sample tube to prepare a thin lipid film on the inner surface of the tube. A degassed phosphate buffer solution (pH 7.0, 20 mL) was then added to the tube. The liposome suspension (1.0 wt %) was prepared by sonication (Tomy Seiko UR-200P) at 60 W for 10 min under a nitrogen atmosphere.[17] The freshly prepared liposome suspensions were incubated at 4 °C overnight to minimize

0024-9297/87/2220-0929$01.50/0

structural defects of the molecular packing in the liposomes.

Polymerization of DODPC lipids as liposomes was carried out as follows:

(A) A total of 0.200 g of lipids was dissolved in chloroform with 2.1 mg of AIBN (5 mol % to the monomeric lipids) and was slowly evaporated in a rotated sample tube. Degassed phosphate buffer solution (pH 7.0, 20 mL) was added to the tube. The liposome suspension was prepared by the same manner as mentioned above. The DODPC liposomes were polymerized for 12 h at 60 °C. The liposomes containing different amounts of AIBN were also prepared in the same manner. A small amount of AIBN was revealed to be decomposed by the 20-min sonication at 60 W. It was almost impossible to prepare the monomeric liposomes containing effective AIBN with less than 0.5 mol % to the DODPC by sonication. The monomeric liposomes containing a small amount of AIBN should be prepared with care. One of the important points in preparing liposomes with an accurate amount of AIBN is to prepare the liposomes as relatively concentrated suspension. Liposomes were incubated at 4 °C for 2 h and polymerized under a nitrogen atmosphere at 60 °C. The polymerization conversion was successively analyzed by UV spectrometry.

(B) A liposome suspension was prepared by the same method as mentioned above. AAPD, 5 mol % to the monomeric lipids, was added to the suspension and resonicated for 10 s to put AAPD into the inner aqueous phase of the monomeric liposome. Then the liposomes were polymerized at 60 °C for 12 h. The polymerization conversion was also determined successively by UV spectrometry.

(C) A liposome suspension (1.0 wt %) containing no radical initiator was put into a quartz tube, sealed under a nitrogen atmosphere, and set in a water bath at a distance of 5 cm from the UV light source (low-pressure mercury lamp; Riko UVL-32W). The liposomes were polymerized by UV irradiation, and the polymerization conversion was checked successively by the spectral change at 255 nm.

A liposome suspension was periodically pipeted out from the sample tube during polymerization. A diluted sample solution was analyzed by UV spectrometry to quantitatively analyze the polymerization conversion. The molar extinction coefficient at 255 nm, corresponding to the diene groups, was successively analyzed to determine the polymerization conversion for DODPC.

The average molecular weight of DODPC lipids, polymerized as liposomes by UV irradiation, was measured by a vapor pressure osmometer (Hitachi Type-117) with temperature control apparatus. The polymerized liposome suspension was dropped on copper grids and they were stained by uranyl acetate. The sample grids were observed to confirm their bilayer structure by TEM (JEOL-100CX). The polymerized liposome suspension was freeze-dried and the obtained dry DODPC polymers were characterized by mass spectrometer (JEOL JMS-DX300). Monomeric lipids were also checked as reference.

A liposome suspension with a concentration of 0.10 wt % was set in a round cell with a diameter of 10 mm. A phosphate buffer solution of Triton X-100 was added stepwise to the liposome suspension, and the scattered-light intensities (90°) were successively recorded with an apparatus (Union Giken LS-601) at 25 °C to evaluate the stability of the polymerized liposomes against surfactant attack. A He–Ne laser with a wavelength of 632.8 nm was used as the light source.

Carboxyfluorescein (CF) was incorporated into the inner aqueous phase of the liposomes with the final concentration of 0.10 mol/L by a similar manner as previously reported.[17] CF-containing liposomes were fractionated and purified by gel permeation chromatography (Sepharose CL-4B). The leakage of CF was followed as the increase of fluorescence intensity at 520 nm by fluorescence spectrometer (Hitachi HPF-4) with an excitation beam of 330 nm. Generally, there was no significant CF leakage from a series of the polymerized liposomes at 25 °C in aqueous solution (pH 7). To accelerate the leakage of CF from the inner aqueous phase of the polymerized liposomes, the pH was preliminarily set to 8.6 (before sonication) and the CF leakage was measured at 50 °C.

Results and Discussion

Polymerization of Monomeric Lipids as Liposomes. 1,2-di(2,4-octadecadienoyl)-3-phosphatidylcholine (DODPC) was dispersed in a degassed phosphate buffer solution and small unilamellar liposomes were prepared by sonication under N_2 atmosphere. The dispersion was then UV-irradiated to polymerize the liposomes. Solution was periodically pipeted out to determine the polymerization conversion by UV absorption at 255 nm. The absorption at 255 nm, corresponding to diene groups, disappeared with the UV irradiation. The conversion curve is depicted from the decrease of this absorption as shown in Figure 1. The 3-h UV irradiation provided almost 100% polymerization conversion. Against this, however, azobis(isobutyronitrile) (AIBN) or azobis(2-amidinopropane) dihydrochloride (AAPD) also induced radical polymerization of DODPC as liposomes; their polymerization conversion reached aroung 50–60% as shown in Figure 1. Further incubation up to 12 h did not induce further polymerization except for the contribution of thermal polymerization. The polymerization conversion of DODPC liposomes polymerized by only heating for 12 h without radical initiators was about 5–10%. The polymerization conversion was almost independent of the concentration of these initiators as long as they were polymerized by either AIBN or AAPD.[18]

When AIBN and AAPD were introduced into a hydrophobic region and an aqueous phase, respectively, to initiate the radical polymerization, the polymerization conversion clearly improved and reached about 95% by heating at 60 °C, as shown in Figure 2. The polymerization conversion was also improved by the heating at 60

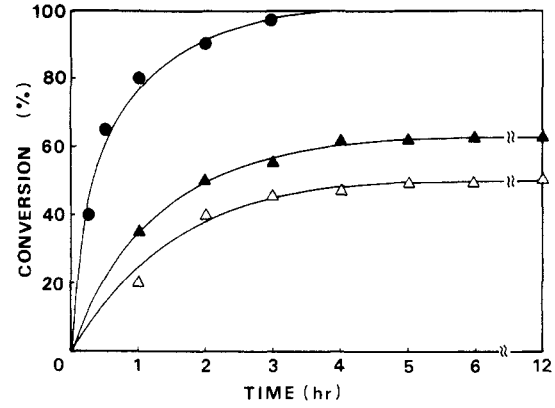


Figure 1. Polymerization conversion of DODPC liposomes. DODPC liposomes (1.00 wt %) were polymerized by UV irradiation at 20 °C (●), by AIBN (△), or by AAPD (▲) at 60 °C. The conversion was determined by the decrease of the absorption at 255 nm corresponding to the diene groups.

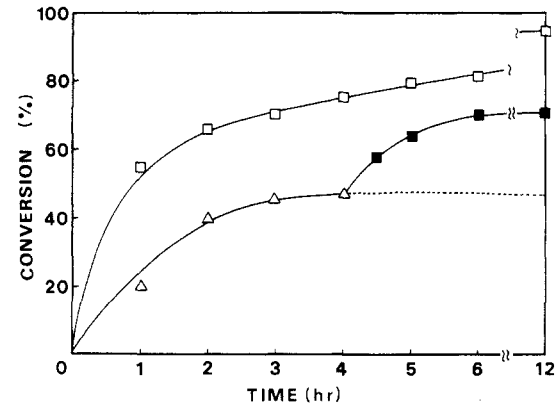


Figure 2. Polymerization conversion of DODPC liposomes. DODPC liposomes were polymerized by AIBN and AAPD simultaneously at 60 °C (□), or by the addition of AAPD to the DODPC liposomes preliminarily polymerized by AIBN at 60 °C (■).

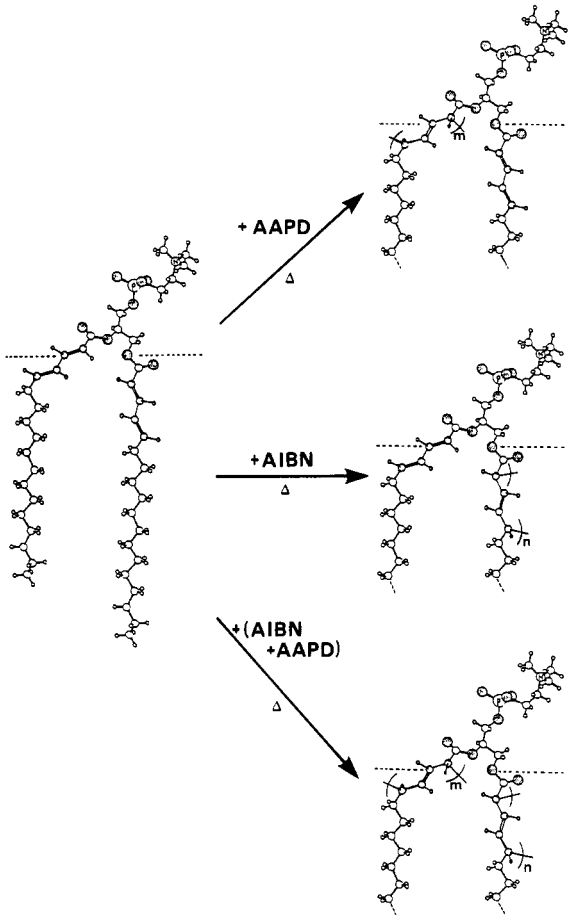


Figure 3. Schematic representation of the DODPC molecule as part of the liposomes and possible attack with radical initiators.

°C after the addition of AAPD to the liposomes that were preliminarily polymerized by AIBN, as shown in Figure 2. These results pushed us to speculate the polymerization mechanism of DODPC liposomes. Namely, AIBN and AAPD were strongly suggested to initiate the radical polymerization of two different diene groups in 1- and 2-acyl chains. The acyl chain packing for choline-type lipids in the bilayer membrane has already been discussed by Seelig et al.[19] and Pearson et al.[20] It has also been summarized by Hauser et al.[21] They emphasized that the glycerol segment was perpendicularly oriented to the 2-dimensional direction of bilayer membrane. Seelig and Seelig have also distinguished the segmental motion of two acyl chains in liposomes.[19] So, it is not difficult to reach the following hypothesis that the two diene groups have different locations and therefore have a different chemical environment in the liposomes. The unequivalent characteristics of the diacetylene groups in 1- and 2-acyl chains of the diacetylene phosphocholine-type lipid have already been discussed by O'Brien et al.[22] and Chapman et al.[23] Especially, O'Brien et al. expected a different polymerization reaction between diacetylene groups in 1- and 2-acyl chains from their different chain packing;[22] i.e., two acyl chains did not extend equally into the lipid bilayer. This means that the diacetylene phosphocholine-type lipids have no ability to undergo intramolecular polymerization. In their diacetylene phospholipid liposomes, the two diacetylene groups could not be distinguished by the manner of polymerization because the diacetylene groups in both the 1- and 2-acyl chains were located deep in the hydrophobic region and there was no effective method to initiate their selective polymerization. In this DODPC lipid, the diene group was located in the 2,4-position, near the hydrophilic region. The unequivalent characteristics of these diene groups in 1- and 2-acyl chains were considered to appear by the acyl chain packing in liposomes.

Table I
Characteristics of the Polymerized DODPC as Liposomes

expt	polymerization method	time, h	temp, °C	$\bar{M}_w$	solubility[a]
1	UV	6	0	5200	soluble
2	UV	2	20	4500	soluble
3	UV	2	50	4500	soluble
4	AIBN	4	60		partially
5	AAPD	4	60		partially
6	AAPD + AIBN	6	60		insoluble

[a] In methanol, ethanol, and chloroform.

It is not very difficult to speculate that chain propagation polymerization occurred in a different direction during polymerization initiated by AIBN and AAPD simultaneously to prepare a tight network structure. The reactivity of the diene groups in 1-acyl chains was probably independent of that of the diene groups in 2-acyl chains. A possible polymerization is schematically depicted in Figure 3. The general idea of packing of lipids in bilayer membranes provided the different chemical environments surrounding the two different diene groups bound to 1- and 2-acyl chains. The diene groups in 2-acyl chains might be exposed to the aqueous phase, and therefore polymerization was easily initiated by a water-soluble radical initiator, AAPD. Two diene groups in one lipid molecule separated from each other when oriented to form a bilayer structure, so that it is almost impossible to polymerize these diene groups intramolecularly without disturbing the molecular packing suitable for the bilayer structure. As AIBN is a hydrophobic radical initiator, it should be dissolved in the hydrophobic region of the bilayer. This radical might attack the diene groups in the 1-acyl chains and initiate the polymerization intermolecularly. On the other hand, if both AIBN and AAPD were added into a hydrophobic region and an aqueous phase, respectively, AIBN and AAPD would independently attack and initiate the intermolecular radical polymerization of the 1- and 2-acyl chains, respectively, to form a tight polymerized membrane structure.

Membrane Stability of the Polymerized Liposomes. The freeze-dried DODPC lipids polymerized as liposomes by UV irradiation were soluble in some organic solvents such as methanol.[24] The average molecular weight of DODPC, polymerized by UV irradiation, was determined by vapor pressure osmometry as about 5200 as shown in Table I. Namely, the UV irradiation might provide only oligomers of DODPC when they were polymerized as liposomes at any temperature. On the other hand, there was no effective organic solvent to solubilize the freeze-dried DODPC liposomes which were polymerized by AIBN, AAPD, or both.[25] This suggested that radical initiators such as AIBN or AAPD provided polymeric derivatives with a relatively higher average molecular weight. There was no conventional method to measure the molecular weight because there was no effective solvent for them. Mass spectrometry was applied to analyze the molecular profile of DODPC polymerized by UV irradiation or radical initiators. The freeze-dried DODPC liposomes completely polymerized by UV irradiation gave fragment ion peaks at m/e 263, 279, 599, etc., which were the same as those of monomeric DODPC lipids. This means that some of the UV-polymerized DODPC molecules have the same acyl chains as those of the monomeric state. As the degree of polymerization was calculated only by the UV spectral intensity at 255 nm, which corre-

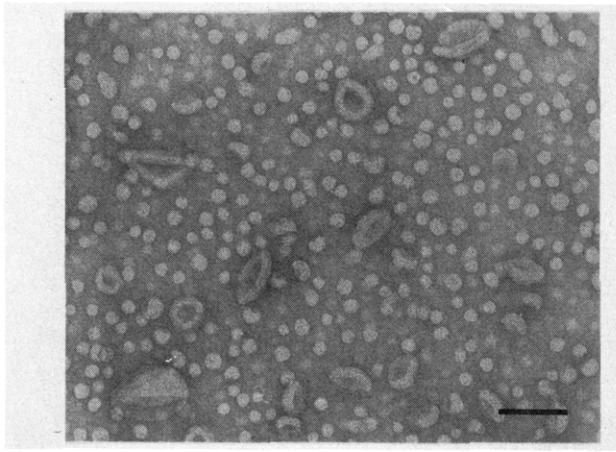


Figure 4. Transmission electron micrograph of typical DODPC liposomes polymerized by both AIBN and AAPD simultaneously. Liposomes were stained by uranyl acetate. Space bar is 2000 Å.

sponded to the diene structure, isomerization of the diene structure can also be detected as the polymerization. The result from mass spectrometry suggested the inefficient polymerization of DODPC by UV irradiation. The lyophilized DODPC lipids polymerized as liposomes by radical initiators were hard to detect by mass spectrometry, but they did not show any fragment ion peaks corresponding to those for monomeric lipids. This also supported the formation of a tight structure of liposomes that were polymerized by radical initiators.

The bilayer structure of the polymerized liposomes was confirmed by TEM. (A typical TEM micrograph of DODPC liposomes polymerized by AIBN is shown in Figure 4. From the TEM pictures, it was also suggested that the polymerization of DODPC lipids initiated by AIBN and/or AAPD did not affect seriously the molecular packing for the bilayer liposomes.

Carboxyfluorescein (CF) was frequently used to evaluate the leakage of the entrapped water-soluble materials in the inner aqueous phase of the liposomes. CF-containing DODPC liposomes were prepared by the same method as previously reported[17] and were polymerized by the above-mentioned methods. CF-containing polymeric DODPC liposomes were fractionated and purified by a Sepharose CL-4B column. To accelerate the CF leakage from the inner aqueous phase of the polymerized liposomes, the pH of the liposome suspension was preliminarily set to 8.6 before sonication. The all experiments have also been taken at 50 °C also to accelerate the leakage. As we mentioned in our previous paper,[17] DODPC liposomes showed less entrapping ability (higher leakage) when they were polymerized by UV irradiation. About 90% of the incorporated CF leaked within 60 min. Against this, DODPC liposomes polymerized by AIBN and/or AAPD showed excellent entrapping efficiency as shown in Figure 5. Especially, DODPC liposomes, polymerized by AIBN and AAPD simultaneously, showed the best stability as expected above.

The effect of surfactant on the bilayer structural change is also a good parameter to evaluate the membrane stability of liposomes. The scattered-light intensity at 90° was conventionally used to check the aggregation and destruction of liposomes. Triton X-100 is known as a surfactant strong enough to destroy a bilayer structure and to extract proteins from biological membranes. Monomeric DODPC liposomes showed typical aggregation and fusion as ordinary lipid liposomes that were induced by the addition of Triton X-100. As shown in Figure 6, the scattered-light intensities from the suspension increased and

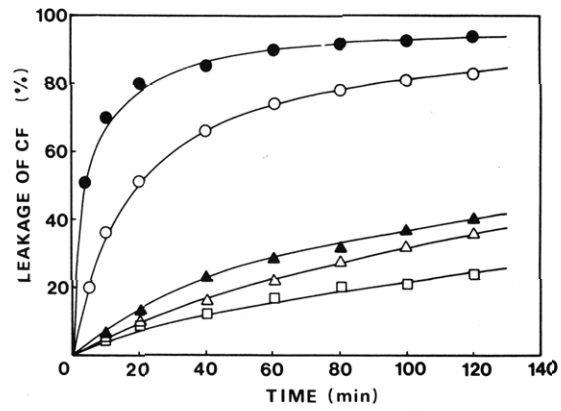


Figure 5. Leakage of CF from the inner aqueous phase of the liposomes polymerized by UV irradiation (●), AIBN (△), AAPD (▲), or AIBN and AAPD simultaneously (□). Leakage was measured at 50 °C. The liposome suspension was preliminarily set at pH 8.6 to accelerate the leakage. The 100% CF leakage was performed by the sonication at 50 W for 5 min in the presence of Triton X-100.

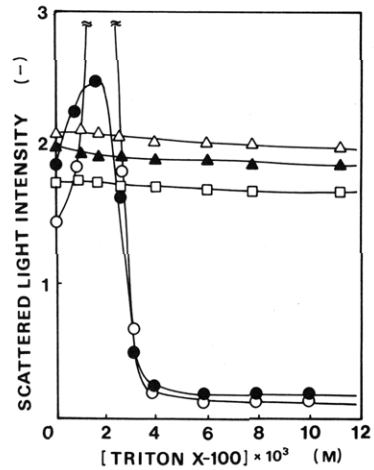


Figure 6. Scattered-light intensity changes for polymerized DODPC liposomes by the addition of Triton X-100 at 25 °C. Liposomes were monomeric (○) or polymerized by UV irradiation (●), AIBN (△), AAPD (▲), or AIBN and AAPD simultaneously (□).

decreased by the continuous addition of detergent which corresponded to the aggregation and destruction of liposomes, respectively. DODPC liposomes preliminarily polymerized by UV irradiation were also destroyed by the addition of Triton X-100 in the same manner as for monomeric ones. Liposomes polymerized by AIBN and/or AAPD did not show any significant intensity changes as shown in Figure 6. It is easy to understand that the polymerized network structure prevents the insertion of the hydrophobic alkyl chain of Triton X-100 into the hydrophobic region of the bilayer. A hydrophilic ethylene oxide unit also induced the aggregation of liposomes. Poly(ethylene oxide) is also known to interact with liposomes.[26] For example, poly(ethylene oxide) expels the water molecules facing the hydrophobic region near the hydrophilic membrane surface[27] and stabilizes the system entropically, which induces the aggregation and/or fusion of liposomes.[28] In the polymeric liposome systems except for UV irradiated ones, every lipid molecule was covalently bonded to each other. This might inhibit the interaction between the liposome surface and the ethylene oxide unit; therefore the scattered-light intensity from the polymeric liposome suspension was not changed by the addition of Triton X-100. It should be noted that the instability of the UV-polymerized DODPC liposomes deeply depended on the

photopolymerization condition. It is possible to prepare more stable liposomes by UV irradiation, as Ringsdorf and his co-workers reported.[29] The point is that a slower photopolymerization seemed to provide more stable liposomes which was strongly related to the power of UV light, irradiation distance, etc.

It was clarified that the polymerization profile of DODPC as liposomes depended on the initiation method, and there was a considerable difference in the stability of the polymerized liposomes because of their different structure. It is quite interesting and significant to select a suitable polymerization method for the construction of stable polymeric liposome systems.

Acknowledgment. This work was partially supported by a Grant-in-Aid from the Ministry of Education, Culture and Science, Japan.

Registry No. DODPC (homopolymer), 107173-12-6; DODPC, 107173-11-5.

References and Notes

(1) Gregoriadis, G., Ed. *Liposome Technology*; CRC Press: Boca Raton, FL, 1984; and references therein.
(2) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley-Interscience: New York, 1982; and references therein.
(3) Regen, S. L.; Czech, B.; Singh, A. *J. Am. Chem. Soc.* **1980**, *102*, 6638.
(4) Johnson, D. S.; Sanghera, S.; Pons, M.; Chapman, D. *Biochim. Biophys. Acta* **1980**, *602*, 57.
(5) Hub, H. H.; Hupfer, B.; Koch, H.; Ringsdorf, H. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 938.
(6) O'Brien, D. F.; Whitesides, T. H.; Klingbiel, R. T. *J. Polym. Sci., Polym. Lett. Ed.* **1981**, *19*, 95.
(7) Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 305.
(8) Hupfer, B.; Ringsdorf, H.; Schupp, H. *Makromol. Chem.* **1981**, *182*, 247.
(9) Akimoto, A.; Dorn, K.; Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 90.
(10) Tundo, P.; Kippenberger, D. J.; Klahn, P. L.; Prieto, N. E.; Jao, T. C.; Fendler, J. H. *J. Am. Chem. Soc.* **1982**, *104*, 456.
(11) Hasegawa, E.; Matsushita, Y.; Eshima, K.; Nishide, H.; Tsuchida, E. *Makromol. Chem., Rapid Commun.* **1985**, *5*, 779.
(12) Babilis, D.; Dais, P.; Margaritis, L. H.; Paleos, C. M. *J. Polym. Sci., Polym. Chem. Ed.* **1985**, *23*, 1089.
(13) Buschl, R.; Folda, T.; Ringsdorf, H. *Makromol. Chem., Suppl.* **1984**, *6*, 245.
(14) Regen, S. L.; Shin, J.-S. *J. Am. Chem. Soc.* **1984**, *106*, 5756.
(15) Ohno, H.; Takeoka, S.; Tsuchida, E. *Polym. Bull. (Berlin)* **1985**, *14*, 487.
(16) Nome, R.; Reed, W.; Politi, M.; Tundo, P.; Fendler, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 8086.
(17) Ohno, H.; Ogata, Y.; Tsuchida, E. *J. Polym. Sci., Polym. Chem. Ed.* **1986**, *24*, 2959.
(18) There is a concentration dependence when radical initiators with a concentration of less than about 3 mol % to the lipids were used. A quite small amout of radical initiator, for example, AIBN with 0.1 mol % of monomeric lipids, did not initiate the radical polymerization because these initiators have already been decomposed by the sonication (see Experimental Section).
(19) Seelig, A.; Seelig, J. *Biochemistry* **1974**, *13*, 4839; *Biochim. Biophys. Acta* **1975**, *406*, 1.
(20) Pearson, R. H.; Pascher, I. *Nature (London)* **1979**, *281*, 499.
(21) Hauser, H.; Pascher, I.; Pearson, R. H.; Sundell, S. *Biochim. Biophys. Acta* **1981**, *650*, 21.
(22) Lopez, E.; O'Brien, D. F.; Whitesides, T. H. *J. Am. Chem. Soc.* **1982**, *104*, 305.; *Biochim. Biophys. Acta* **1982**, *693*, 437.
(23) Pons, M.; Villaverde, C.; Chapman, D. *Biochim. Biophys. Acta* **1983**, *730*, 306.
(24) The lyophilized DODPC liposomes polymerized by UV irradiation dissolved rapidly in several organic solvents such as methanol, ethanol, and chloroform. As in those solvents, no bilayer liposomes structure was observed by laser-light scattering, proton NMR, and electron microscopy; the obtained polymerized DODPC was considered to be dissolved homogeneously (monomolecularly) in those solutions.
(25) The DODPC liposomes polymerized by AIBN and AAPD simultaneously actually showed excellent stability against organic solvents. The giant liposomes of DODPC with an average radius of about 1 μm, polymerized by both AIBN and AAPD, were easily analyzed by SEM and there was no change in the liposomal structure after chloroform washing, for example.
(26) Ohno, H.; Maeda, Y.; Tsuchida, E. *Biochim. Biophys. Acta* **1981**, *642*, 27.
(27) Cabane, B. *J. Phys. Chem.* **1977**, *81*, 1639.
(28) Ohno, H.; Sakai, T.; Tsuchida, E.; Honda, K.; Sasakawa, S. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 426.
(29) Hupfer, B.; Ringsdorf, H.; Schupp, H. *Chem. Phys. Lipids* **1983**, *33*, 355.