STY and MMA, with respect to head addition of the benzoyloxy radical, can be regarded as another consequence of the steric hindrance caused by the double substitution at the carbon atom at the head of the molecule of MMA. Solomon and co-workers,[20,21] using a procedure involving a radical scavenger, concluded that tail addition of the benzoyloxy radical to MMA at 60 °C occurs about 13 times as frequently as head addition. An earlier report,[22] that head addition is dominant, was shown to be in error and the conclusion[23] was reached that there is almost exclusively tail addition of $C_6H_5COO^\bullet$ to MMA.

It is still not clear why STL and related compounds are so effective in capturing benzoyloxy radicals. It appears that the presence of an aromatic grouping attached directly to each of the carbon atoms of the ethylenic bond is necessary for high reactivity toward the radical; thus 1,3-diphenylpropene (DPP) has a reactivity considerably less than that of STL but comparable to that of STY.[19] Similarity between DPP and STY is expected since the radicals $C_6H_5COOCH(CH_2C_6H_5)CH(C_6H_5)^\bullet$ and $C_6H_5COOCH_2CH(C_6H_5)^\bullet$ are most probably stabilized to about the same extent. Attachment of the benzoyloxy radical to STY can occur at either end of the ethylenic bond although formation of $C_6H_5COOCH_2CH(C_6H_5)^\bullet$ is greatly preferred.[10] On the other hand, the two ends of the double bond in STL must be equally reactive; on that basis, the reactivity of STL toward $C_6H_5COO^\bullet$ could be expected to be twice that of STY whereas it is found that the reactivities differ by a factor of about 6.

Acknowledgment. Support from the Science and Engineering Research Council for use of the 400-MHz NMR facility at the University of Warwick is gratefully acknowledged; Drs. O. W. Howarth and A. Harrison are thanked for their assistance.

Registry No. (*E*)-Biph-STL, 2039-69-2; (*E*)-Anth-STL, 42196-97-4; Naph-STL, 2043-00-7; Phen-STL, 1895-98-3; BPO, 94-36-0; (MMA)(BPO) (copolymer), 80-62-6; STY, 100-42-5.

References and Notes

(1) Bevington, J. C.; Huckerby, T. N. *Macromolecules* **1985**, *18*, 176-178.
(2) Barson, C. A.; Bevington, J. C. *Eur. Polym. J.* **1987**, *23*, 251-253.
(3) Bevington, J. C.; Breuer, S. W.; Huckerby, T. N. *Angew. Makromol. Chem.* **1986**, *140*, 145-152.
(4) Weale, K. E. *Polymer* **1987**, *28*, 2151-2156.
(5) Bevington, J. C.; Breuer, S. W.; Huckerby, T. N. *Polym. Bull. (Berlin)* **1984**, *12*, 531-534.
(6) Bevington, J. C.; Huckerby, T. N.; Liu, M. G.; Thorpe, F. G. *Eur. Polym. J.* **1987**, *23*, 95-97.
(7) Drefahl, G.; Ponsold, K. *Chem. Ber.* **1960**, *93*, 472-481.
(8) Everett, J. L.; Kon, G. A. R. *J. Chem. Soc.* **1948**, 1601-1603.
(9) Bergmann, E. D.; Bergmann, F. *J. Am. Chem. Soc.* **1937**, *59*, 1443-1450.
(10) Moad, G.; Solomon, D. H.; Johns, S. R.; Willing, R. I. *Macromolecules* **1982**, *15*, 1188-1192.
(11) Bevington, J. C.; Brooks, C. S. *Makromol. Chem.* **1958**, *28*, 173-175.
(12) Bevington, J. C.; Johnson, M. *Makromol. Chem.* **1967**, *102*, 73-82.
(13) Bevington, J. C.; Huckerby, T. N.; Vickerstaff, N.; Barson, C. A. *Polymer* **1986**, *27*, 1823-1825.
(14) Rembaum, A.; Eisenberg, A. *Macromol. Rev.* **1967**, *1*, 57-90.
(15) Bevington, J. C.; Ebdon, J. R. In *Developments in Polymerisation—2*; Haward, R. N., Ed.; Applied Science: London, 1979; Chapter 1.
(16) Bevington, J. C.; Ayrey, G. *Eur. Polym. J.* **1987**, *23*, 497-499.
(17) Ballesteros, J.; Howard, G. J.; Teasdale, L. *J. Macromol. Sci., Chem.* **1977**, *A11*, 29-37.
(18) Ayrey, G.; Bevington, J. C. *J. Macromol. Sci., Chem.* **1985**, *A22*, 229-234.
(19) Barson, C. A.; Bevington, J. C.; Breuer, S. W.; Huckerby, T. N., to be submitted for publication.
(20) Cuthbertson, M. J.; Moad, G.; Rizzardo, E.; Solomon, D. H. *Polym. Bull. (Berlin)* **1982**, *6*, 647-651.
(21) Moad, G.; Rizzardo, E.; Solomon, D. H. *Aust. J. Chem.* **1983**, *36*, 1573-1588.
(22) Kamachi, M.; Kuwae, Y.; Nozakura, S. *Polym. Bull. (Berlin)* **1981**, *6*, 143-146.
(23) Kunitake, T.; Murakami, S. *J. Polym. Sci., Polym. Chem. Ed.* **1974**, *12*, 67-81.

Effect of Phase Transition on Photosensitized Radical Polymerization of Diene-Containing Lipids as Liposomes[1]

Hiroyuki Ohno, Shinji Takeoka, Hidetaka Iwai, and Eishun Tsuchida*

Department of Polymer Chemistry, Waseda University, Tokyo, 160 Japan.
Received January 4, 1988

ABSTRACT: 1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) was polymerized in liposomes by organic free radicals which were generated by photosensitized cleavage of the added azo-type radical initiators such as azobis(isobutyronitrile) (AIBN) or azobis(2-amidinopropane) dihydrochloride (AAPD). Selective polymerization of diene groups on the 1- or 2-acyl chain of DODPC was carried out with water-insoluble AIBN or water-soluble AAPD, respectively, at any temperature, even at temperatures lower than the phase transition temperature (T_c) of the DODPC liposomes. The diene group in the 1-acyl chain locates in the hydrophobic region of the bilayer, whereas that in the 2-acyl chain faces an aqueous phase at any temperature. When AAPD was added to the outer aqueous phase of DODPC liposomes, polymerization conversion initiated by the photosensitized AAPD radicals reached around 50% above the T_c but it remained 27% below the T_c. This confirms that AAPD added to the outer aqueous phase of the liposome can penetrate the lipid membrane above the T_c, but not below it. The liposomes which were polymerized in either the outer or the inner half of the bilayer membrane were therefore prepared by applying this penetration profile. The liposomes polymerized only in the outer layer were as stable as those fully polymerized. The initial polymerization rate of DODPC initiated by photosensitized AIBN radicals in the lipid membrane also reflected the membrane viscosity. Lower polymerization conversions, reflecting considerable recombination of free radicals, were found below T_c due to higher membrane microviscosity.

Introduction

Phospholipid liposomes are being applied as microcapsules for carriers of drugs or functional particles as well as models for biomemranes.[2] These liposomes are, however, not very stable and generally cause aggregation and fusion. Polymerization of monomeric lipids is considered to be a potential technique to stabilize the lipid assemblies.[3,4] The polymerized lipid liposomes actually provide stable microcapsules in some cases, but we have already warned that the membrane stability deeply depends on

0024-9297/89/2222-0061$01.50/0

the polymerization method.[5] For example, stable liposomes of 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) cannot be obtained by UV-irradiated polymerization because of the formation of relatively short polymer chains. On the other hand, DODPC liposomes polymerized with radical initiators at 60 °C have been revealed to be more stable than those polymerized by UV irradiation. The free radical initiated polymerization is not considered to be a very useful tool to obtain stable liposomes.[5,6] Furthermore, selective polymerization of diene groups on either the 1- or 2-acyl chain of DODPC has been carried out by applying both the unequivalent packing of these acyl chains in the bilayer membrane and the different solubilities of the radical initiators.[5] This unequivalent location of diene groups has been applied to the analyses of molecular packing. Since the radical cleavage of those initiators generally requires heating around 50–60 °C, we can obtain no information about the effect of temperature on the polymerization manner especially at temperatures below the T_c. Instead of heating, UV light (360 nm) irradiation was applied to the cleavage of azo-type radical initiators. In the present paper, the polymerization profile of DODPC as a liposome is discussed in relation to membrane permeability and viscosity in both gel and liquid crystalline states.

Experimental Section

Materials. 1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) was purchased from Nippon Oil & Fats Co., Ltd. 1-Palmitoyl-2-(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (POPC) was a gift from Toyo Soda Co., Ltd. The purity of lipid was confirmed with thin-layer chromatography (Merck, silica gel plates) with chloroform/methanol/water (65:35:5 by volume). Samples showing a single spot with an R_f value of around 0.3 were used for experiments.[6]

Azobis(isobutyronitrile) (AIBN) and azobis(2-amidinopropane) dihydrochloride (AAPD) were purchased from Wako Pure Chemical Industries, Ltd., and were purified by recrystallization twice from methanol and distilled water, respectively.

Methods. A total of 0.2 g of DODPC was dissolved in dehydrated chloroform and was slowly evaporated in a rotating sample tube to prepare thin films on the inner surface of the tube; then 20 mL of distilled water was added. A small unilamellar liposome suspension was prepared by sonication (Tip type, Tomy Seiko UR-200P) at 60 W for 20 min under a nitrogen atmosphere.[6] Freshly prepared liposomes were incubated at 8 °C to increase liposome size and to minimize the disordered molecular packing by liposome fusion.[7,8] AIBN (5.0 mol % to the polymerizable lipids) was added into a chloroform solution of DODPC before preparation of the liposome suspension. AAPD (5.0 mol % to the polymerizable lipids) was added directly to the liposome suspension to avoid decomposition of AAPD by sonication. As AAPD was added in the outer aqueous phase of the liposomes, it was considered that there were no AAPD molecules in an inner aqueous phase of liposomes at gel state. The suspension was sonicated for 10 s to put sufficient AAPD molecules into an inner aqueous phase of the liposomes.[9]

On the other hand, AIBN was incorporated into the hydrophobic region of the liposome membrane by sonication. It was estimated that about 10% of the incorporated AIBN was decomposed by the 20-min sonication.[9] The liposome suspension was put into a Pyrex tube (5-mm i.d.), sealed under a nitrogen atmosphere, and fixed in a thermostated bath with a distance of 5 cm from the UV light source (high-pressure mercury lamp, Riko UVL-100P). The UV light with wavelength shorter than 360 nm was cut with an UV filter (Hoya L-38) to avoid UV-irradiated polymerization of DODPC. The polymerization conversion was successively analyzed by monitoring the decrease of spectral intensity at 255 nm, which was attributed to the diene groups of the polymerizable lipids.[6]

5-Carboxyfluorescein (CF) was incorporated into an inner aqueous phase of the polymerized liposomes (0.10 mol·L^{-1}) by incubation at 60 °C for 100 h.[10] Then the CF-containing liposome suspension was passed through a gel permeation chromatography column (Sepharose CL-4B, 30-mm i.d., 600-mm height) to expel an excess of CF molecules dissolved in an outer aqueous phase. The leakage of CF was detected as the increase of fluorescence intensity at 520 nm by fluorescence spectrometry (JASCO FP-550) with an excitation beam at 330 nm. Liposomes were broken down to release 100% CF by sonication in the presence of Triton X-100. The fluorescence intensity at this stage was used as 100% CF leakage.

The liposome suspension with a concentration of 0.1 g/100 cm^3 was set in a round cell with a diameter of 10 mm. Triton X-100 was added to the solution, and the scattered light intensity (90°) was successively recorded with an apparatus (Union Giken LS-601) at 25 °C to evaluate membrane stability of the polymerized liposomes against the attack of Triton X-100. A He–Ne laser with wavelength of 632.8 nm was used as a light source.

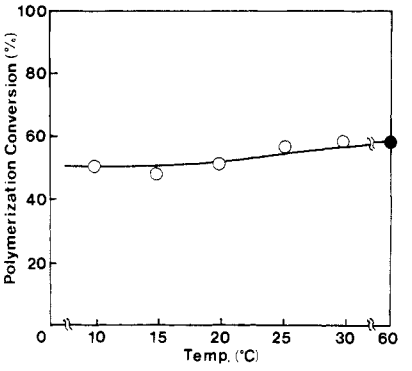


Figure 1. Polymerization conversion of DODPC liposomes after 12 h. Polymerization was initiated by photosensitized AAPD radicals. AAPD molecules were incorporated in both an inner and outer aqueous phase of the liposomes by sonication. Polymerization initiated by AAPD radicals generated at 60 °C (●) is also depicted as a reference.

Results and Discussion

An azobis(2-amidinopropane) dihydrochloride (AAPD), a water-soluble radical initiator, was added to the premade liposome suspension to avoid decomposition of AAPD during liposome preparation by sonication (20 min, 60 W).[5] As liposomes thus prepared were considered to have no AAPD molecule in an inner aqueous phase, they were resonicated for 10 s to incorporate AAPD molecules in both aqueous phases. A photosensitized polymerization was then carried out with irradiation of UV light of 360 nm, necessary for a cleavage of azo bond. Under the same condition, a generation rate of AAPD radicals is independent of the polymerization temperature because the activation energy for the photosensitized radical cleavage is zero. Polymerization of diene-containing phospholipids was completed within 12 h, and polymerization conversion reached 50%. Figure 1 shows the temperature dependence of polymerization conversion (after 12 h) of DODPC liposomes initiated by AAPD radicals. Polymerization conversion of about 50% was found at any temperature. A slight increase in the polymerization conversion at relatively higher temperature should be attributed to the contribution of thermal polymerization. This 50% conversion has already been attributed to the polymerization of diene groups in only the 2-acyl chains for DODPC.[5] It indicates that a geometric location of diene groups on both acyl chains is maintained at any temperature or changes only a little, which cannot be detected through this polymerization profile. The selective radical polymerization of DODPC is observed even at low temperatures with photosensitized polymerization. This method is considered to be useful to polymerize liposomes, especially for the liposomes incorporating some chemicals or enzymes that are easily denaturated or decomposed by heating.

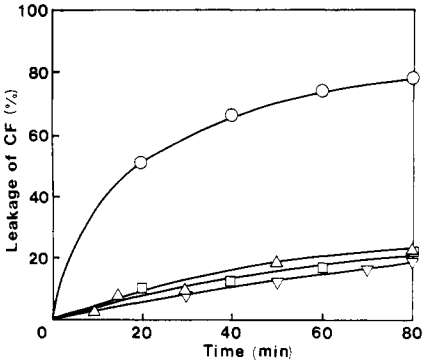


Figure 2. Leakage of CF from an inner aqueous phase of polymerized DODPC liposomes at 50 °C. Liposomes were polymerized by photosensitized AAPD radicals at 10 °C (▽) or 30 °C (△) or by AAPD radicals generated at 60 °C (□). That of monomeric liposomes (○) was also depicted as a reference.

A leakage of carboxyfluorescein (CF) was frequently measured to evaluate membrane stability of thus polymerized liposomes.[4,5] Figure 2 shows the CF leakage from DODPC liposomes polymerized with photosensitized AAPD radicals at 10 and 30 °C. Those from monomeric and polymerized liposomes with AAPD at 60 °C are also depicted as references. The leakage measurement was carried out at 50 °C to accelerate the CF leakage.[5] The CF leakage from DODPC liposomes is considerably restricted after photosensitized radical polymerization. There is little difference in CF release among liposomes polymerized with photosensitized AAPD at 10 and 30 °C, and even those with thermally cleaved AAPD at 60 °C. The polymerized DODPC liposomes with excellent stability are obtained through the polymerization with these initiator radicals cleaved by UV light. It was concluded that the radical polymerization was a very effective method to prepare stable liposomes from diene-containing phospholipids at any temperature.

Photosensitized polymerization of only outer facing DODPC lipids was carried out for liposomes without resonication to keep all of the AAPD molecules in only an outer aqueous phase of the liposomes. The temperature dependence of the polymerization conversion was also examined and the results are shown in Figure 3 (○). As expected by the temperature dependence of the permeation profile through a membrane,[11] the polymerization profile clearly reflected the phase transition behavior. Polymerization conversion after 12 h was 27% when the polymerization temperature was below the phase transition temperature (T_c = 18 °C by DSC), and it reached about 50% above the T_c. The polymerization conversion above the T_c was the same as that obtained from the photosensitized polymerization after resonication. It showed that AAPD molecules (not radicals) could penetrate through the lipid membrane when the temperature was higher than the T_c, and AAPD could attack diene groups of the 2-acyl chains for DODPC in both outer and inner halves of the bilayer of liposomes. Penetration of AAPD had already been reported for the radical polymerization of 1-palmitoyl-2-(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (POPC) as liposomes at 60 °C.[9] All of diene groups of the 2-acyl chains for POPC were polymerized by simple addition of AAPD to the liposome suspension when polymerization was carried out at temperature above T_c.

The average radius of the liposomes used for this experiment was about 40 nm. These unilamellar liposomes were obtained by incubation (8 °C for 12 h) of small unilamellar liposomes prepared with sonication. The average radius was calculated by the transmission electron microscopy and the ^{1}H NMR signal intensity ratio for choline

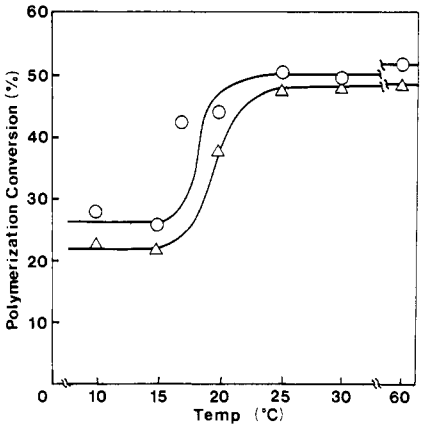


Figure 3. Polymerization conversion of DODPC liposomes initiated with photosensitized AAPD radicals. AAPD molecules were incorporated in an outer aqueous phase only by simple addition of AAPD to a liposome suspension at temperatures below T_c (○) and in an inner aqueous phase only by expelling AAPD in an outer aqueous phase of liposome suspension by column chromatography (△).

methyl protons split by Eu^{3+}. A ratio of the numbers of lipids in outer monolayer to that in the inner one is about 54:46, and AAPD can attack diene groups of 2-acyl chains for DODPC. Below the phase transition temperature, however, only diene groups of 2-acyl chains for DODPC in the outer half of the bilayer were polymerized by AAPD because AAPD molecules could not penetrate through lipid membrane at this condition. AAPD in an outer aqueous phase of the liposome (average radius = 40 nm) can therefore attack 27% of the total diene groups in the ideal case. This is in good agreement with the experimental data (27%). It was concluded that AAPD was able to pass through the bilayer membrane above the T_c, but not below it. The polymerization of the liposome is clearly shown to reflect the membrane permeability of radical initiators through the liposomes.

The membrane permeability changes at the phase transition temperature have hitherto been analyzed with techniques such as leakage measurement of a fluorescent probe from an inner aqueous phase of liposomes.[10] This method is useful to investigate the amount of rapidly released CF. The fastest CF release occurred at the phase transition temperature of the liposomes, and CF release was considerably restricted at temperatures below or above the T_c. On the other hand, in the analysis of photosensitized polymerization presented here, the amount of penetrated AAPD cannot be measured because the polymerization conversion obtained does not depend on the leaked amount.

There are three different types of the polymerized liposomes, namely, liposomes polymerized in their inner, outer, or both layers as schematically shown in Figure 4. Liposomes polymerized in only the outer layer (C) were prepared by photosensitized polymerization of liposomes at temperatures lower than T_c. For this, AAPD should be added to the preliminarily prepared liposomes (procedure II). For the preparation of liposomes selectively polymerized in only the inner layer (B), separation of AAPD molecules from the outer aqueous phase by column chromatography and photosensitized polymerization should be carried out below the T_c (procedure I). Ordinary polymerization (procedure III) normally provides liposomes where both layers were polymerized (D) regardless of the location of AAPD molecules. These polymerized liposomes (D) may be obtained from liposomes B with procedure II. Inner polymerized liposomes can be easily obtained by applying the following technique. The AAPD was added

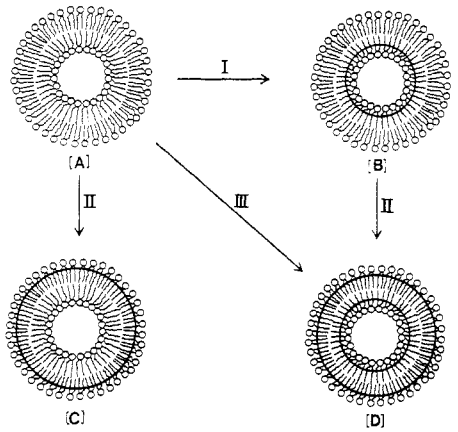


Figure 4. Schematic representation of selective polymerization in liposomes. Monomeric liposomes (A) were selectively polymerized in either the inner (B) or the outer layer (C), or both were polymerized (D). Procedures: (I) AAPD was cosonicated with the liposomes to put AAPD into both outer and inner aqueous phase of the liposomes. The AAPD present in the outer aqueous phase was separated by column chromatography. Photosensitized polymerization was carried out at a temperature below T_c of the liposomes. (II) AAPD was simply added to the liposome suspension. Photosensitized polymerization was then carried out below T_c. (III) AAPD was consonicated with liposomes and then polymerized regardless of the AAPD location.

to a liposome suspension (5 mol % to the total of lipids) and was resonicated for 10 s. This suspension was passed through a gel permeation column (Sepharose CL-4B, 30-mm i.d., 600-mm height) to expel AAPD in an outer aqueous phase. The column was kept at 4 °C to avoid leakage of AAPD from an inner aqueous phase of liposomes. Photosensitized polymerization of DODPC liposomes thus prepared was carried out by the same method as mentioned above. The temperature dependence on the polymerization conversion is also shown in Figure 3 (△). The polymerization conversion of 23% was obtained at temperatures below the phase transition temperature. As the complete polymerization of the 2-acyl chains for DODPC in an inner monolayer should give conversion of 23% for the liposomes with an average radius of 40 nm, inner polymerized liposomes were strongly suggested to be obtained by this method. Especially liposomes having a polymerized inner half of the bilayer (B) are expected to be the model for an erythrocyte membrane. The erythrocyte membrane (ghost membrane) is known to be reinforced with a network structure of spectrin, actin, and ankirin on the inner surface of the membrane. Some basic data of the liposomes having a polymerized inner half of the bilayer is mentioned in this paper. Detailed characteristics will be summarized in the near future. The impermeability of AAPD molecules at lower temperature than T_c is concluded to be useful to prepare the liposomes having the polymerized layer in a different location (outer, inner, or both). Selective polymerization of localized counterion ammonium monomers for phosphatidic acid liposomes has already been carried out to make selective polymer coatings on the either surface of the liposomes.[12] This is a similar experiment to ours. However, characteristics of prepared polymerized liposomes are different.

The stability of the polymerized liposomes against detergent attack was studied by light-scattering measurement (Figure 5). Scattered-light intensities of monomeric DODPC liposomes raised rapidly and decreased gradually with increasing concentration of Triton X-100. Liposomes, where lipids in both sides of the bilayer were polymerized with photosensitized AAPD, showed an excellent stability and no scattered-light intensity change was found. The

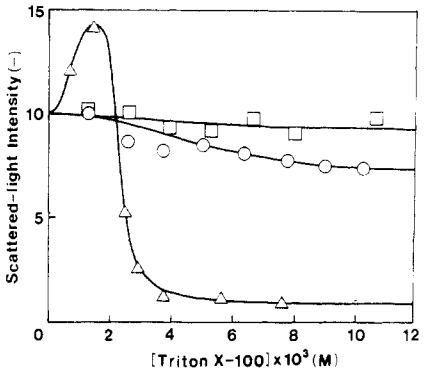


Figure 5. Scattered-light intensity changes for aqueous suspensions of outer polymerized liposomes (○). Those for monomeric (△) and completely polymerized liposomes with photosensitized AAPD at 30 °C were also shown as references (□).

liposomes, composed of a polymerized outer monolayer and a monomeric inner one, were nearly as stable as the completely polymerized liposomes in both layers. This indicated that the essential condition to prepare stable liposomes was to polymerize their outer halves of the bilayer.

As mentioned above, we prepare liposomes having a polymerized outer half of the bilayer, called outer-polymerized liposomes, by applying the impermeability of AAPD at temperatures below the T_c. Tundo et al. have already reported selective polymerization of one side of the bilayer membrane.[13] They unfortunately misunderstood the phenomenon as selective polymerization. They used liposomes from a surfactant having allyl group as part of the polar head group and polymerized them by the addition of AIBN to a liposome aqueous suspension at 60 °C. They suggested the liposomes polymerized the outer half of the bilayer from the resulting only 60% loss of the double bonds. It seems doubtful to us that they really have obtained such liposomes, because water-insoluble AIBN easily invades the bilayer membrane and penetrates the hydrophobic region above T_c and accordingly AIBN attacks polymerizable groups in both sides of bilayer membrane. Furthermore, polymerization of the amphiphile as liposomes seems to be very difficult with AIBN because their polymerizable groups are considered to be exposed to an aqueous medium. Both a water-soluble initiator such as AAPD and polymerization at temperatures below gel-to-liquid crystalline phase transition temperature are necessary for the selective polymerization of the bilayer. Nearly 50% of the polymerization conversion was found about the T_c regardless of the AAPD location at lower temperature than the T_c. From these results, AAPD was confirmed to penetrate the bilayer membrane of liposomes above T_c. The amount of AAPD leakage from the inner aqueous phase should not be the same as that from the outer aqueous phase. Though the concentration of released AAPD from the inner aqueous phase was 100 times lower than that from the outer aqueous phase, polymerization conversion above T_c reached nearly 50%. The polymerization conversion has almost no relation to initiator concentration. The liposomes which contained AAPD molecules in the inner aqueous phase could be prepared at 4 °C. When the solution temperature was raised, a considerable amount of AAPD should be released at the phase transition temperature. Though the concentration of the released AAPD was about 100 times lower, it was sufficient to polymerize diene groups in the 2-acyl chains of the outer facing layer completely, as mentioned above.

The liposomes, which possess a polymerized inner layer and a monomeric outer layer, have both membrane sta-

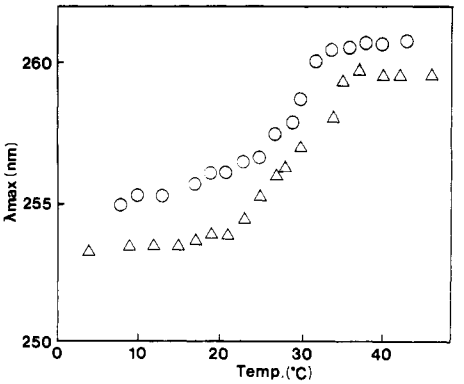


Figure 6. Temperature dependence of the absorption maxima for monomeric DODPC liposomes (△) or inner polymerized DODPC liposomes (○) in an aqueous medium.

bility and mobility. The inner polymerized liposomes were estimated to have the same phase transition temperature as the monomeric ones. We have found that a phase transition temperature of diene-containing lipids as liposomes could be analyzed by λ_{max} for diene groups. The maximum absorbance was found at about 257 nm at temperatures above T_c but it showed a blue shift below T_c.[14] This spectral shift is attributed to the degree of interaction between neighboring diene groups, similar to the stacking of chromophores in the liposomes. This method was applied to analyze the phase behavior of the inner polymerized liposomes. 1-Palmitoyl-2-(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (POPC), which has a 2,4-diene group only in the 2-acyl chain, was used for this experiment because the absorbance of inner polymerized DODPC liposomes originated from unreacted diene groups of both 1- and 2-acyl chains in an outer monomeric layer and from those of 1-acyl chains in an inner polymerized layer. The inner polymerized POPC liposome shows the same phase transition behavior as that of the unpolymerized one (Figure 6). Inner polymerized DODPC liposomes were very stable against heating and cooling. Furthermore, no fusion occurred even after freeze–thawing. CF release from an inner aqueous phase was considerably restricted by the polymerization of only the inner half of the bilayer. All of these data indicate that inner polymerized liposomes are expected to be excellent materials for biomembrane mimetic systems.

AIBN-initiated radical polymerization is also applicable to investigate the phase behavior of liposomes. Water-insoluble AIBN was incorporated as a photosensitizer into the hydrophobic region of the DODPC membrane. Photosensitized polymerization was carried out at low temperatures in a similar way to AAPD polymerization. After 48 h of reaction, the polymerization conversion reached 50%. There was no temperature dependence on the polymerization conversion. All diene groups in the 1-acyl chains of DODPC were considered to be polymerized selectively by an AIBN radical fragment. However, the initial polymerization rate was a function of the photosensitized temperature, especially the change at 16 °C corresponding to the gel-to-liquid crystalline phase transition temperature of DODPC liposomes as shown in Figure 7. To simplify the analysis, polymerization conversion at 30 min after the UV irradiation was used to evaluate the initial polymerization rate. Polymerization conversion after 30-min irradiation was about 3% when temperatures were lower than the T_c. It increased, however, to 8% above the T_c. This indicates that polymerization rate in the crystalline state is smaller than that in liquid crystalline state. In the stage of the initial liposome

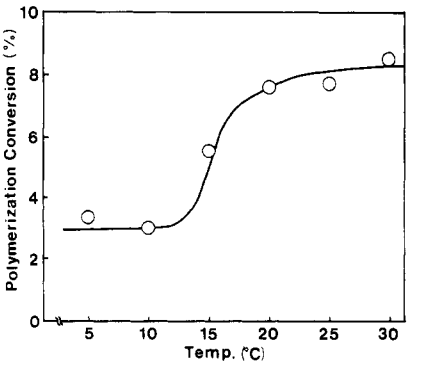


Figure 7. Initial polymerization conversion (after 30 min) of DODPC liposomes initiated with photosensitized AIBN radicals.

polymerization, the lifetime of the propagated individual DODPC radicals seemed to be very small because monomeric lipids were closely packed in the liposome membrane. The rate-determining step of the polymerization was therefore considered to be the initiation reaction. As there is no temperature dependence on the generation of AIBN radicals in photosensitized polymerization, the temperature dependence in Figure 7 is attributed to the frequency of radical attack on the polymerizable lipids. We have already discussed the initial polymerization rate at 60 °C for AIBN- or AAPD-added systems in relation to the initiation reaction.[15] The thermal cleavage rate of AAPD is about six times higher than that of AIBN. However, the initial polymerization rate for the AIBN-added system should be faster than that for the AAPD-added system because the local concentration of AIBN was about 100 times higher than that for AAPD under our experimental conditions. Contrary to this, the actual polymerization rate for the AAPD-added system was larger than that for AIBN system. A lower initiation efficiency for the AIBN-initiated system can be explained by the "cage effect"[16] due to a high local viscosity of the hydrophobic region in the bilayer membrane. The initiation efficiency for AIBN was calculated to be about 0.6–0.7 in ordinary organic solvents, where a diffusion coefficient of a radical pair was about 1×10^{-5} cm^2·s^{-1}. On the other hand, the liposome membrane structure is known to provide a more viscous matrix. For example, Papahadjopoulos et al.[17] reported that the diffusion coefficient of 3,3′-dioctadecyloxocarbocyanine (diO-C_{18}) in dipalmitoylphosphatidylcholine measured with the method of fluorescence recovery after photobleaching was 7.0×10^{-8} cm^2·s^{-1} at 45 °C (above the T_c for DPPC) and less than 5.0×10^{-10} cm^2·s^{-1} at 30 °C (below the T_c for DPPC). In such a viscous medium, the initiation efficiency of AIBN should be smaller and most of the generated radicals should recombine rapidly in the matrix.[18] This explanation was also applied to the photosensitized polymerization profile as shown in Figure 7. Since the diffusion coefficient of AIBN in the gel state was at most by two orders of magnitude smaller than that in the liquid crystalline state, the magnitude of the generation of effective radicals in the gel state was much smaller than that in the liquid crystalline state. This was evidently shown in the results from the initial polymerization rate (Figure 7).

Conclusion

The results of the photosensitized polymerization with water-soluble AAPD clearly reflect the changes in membrane permeability above and below T_c. Applying impermeability of AAPD below T_c, we prepared outer or inner polymerized liposomes. These liposomes possess both stability and fluidity. Initial polymerization rates of

photosensitized polymerization with water-insoluble AIBN provided information about the relative microviscosity of the membrane.

Acknowledgment. We thank Dr. Y. Nagata and Dr. A. Akimoto of Toyo Soda Co., Ltd., for their kind donation of a polymerizable lipid (POPC). This work was partially supported by a Grant-in-Aid for Scientific Research form the Ministry of Education, Science and Culture.

Registry No. DODPC (homopolymer), 108916-62-7; CF, 76823-03-5; POPC (homopolymer), 116698-35-2.

References and Notes

(1) This is part 7 of the series. Part 6: Ohno, H.; Takeoka, S.; Hasegawa, E.; Tsuchida, E. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2007.
(2) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley-Interscience: New York, 1982; and references therein.
(3) Tieke, B. *Adv. Polym. Sci.* **1985**, *71*, 79 and references therein.
(4) Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 305. Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Ibid.* **1988**, *27*, 113 and references therein.
(5) Ohno, H.; Ogata, Y.; Tsuchida, E. *Macromolecules* **1987**, *20*, 929.
(6) Ohno, H.; Ogata, Y.; Tsuchida, E. *J. Polym. Sci., Polym. Chem.* Ed. **1986**, *24*, 2959.
(7) Ohno, H.; Maeda, Y.; Tsuchida, E. *Biochim. Biophys. Acta* **1981**, *642*, 27.
(8) Ohno, H.; Takeoka, S.; Iwai, H.; Tsuchida, E. *Macromolecules* **1988**, *21*, 319.
(9) Ohno, H.; Takeoka, S.; Iwai, H.; Tsuchida, E. *J. Polym. Sci., Polym. Chem. Ed.* **1987**, *25*, 2737.
(10) Ohno, H.; Takeoka, S.; Hayashi, N.; Tsuchida, E. *Makromol. Chem., Rapid Commun.* **1987**, *8*, 215.
(11) Ohno, H.; Ukaji, K.; Tsuchida, E. *J. Colloid Interface Sci.* **1987**, *180*, 486.
(12) Ringsdorf, H.; Schlarb, B. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1986**, *27*(2), 195.
(13) Tundo, P.; Kippenberger, D. J.; Klahn, P. L.; Prieto, N. E.; Jao, T. C.; Fendler, J. H. *J. Am. Chem. Soc.* **1982**, *104*, 456.
(14) Ohno, H.; Takeoka, S.; Iwai, H.; Tsuchida, E. *Bull. Chem. Soc. Jpn.*, in press.
(15) Ohno, H.; Takeoka, S.; Tsuchida, E. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2945.
(16) Noyes, R. M. *J. Am. Chem. Soc.* **1955**, *77*, 2042.
(17) Wu, E.; Jacobson, K.; Papahadjopoulos, D. *Biochemistry* **1977**, *16*, 3936.
(18) DeSchriver, F.; Smets, G. *J. Polym. Sci., Polym. Chem. Ed.* **1966**, *4*, 2201.

Synthesis of Polymerizable and Amphiphilic (Porphinato)irons and Their Copolymers with Polymerizable Phospholipid

Eishun Tsuchida,* Hiroyuki Nishide, Makoto Yuasa, Takeshi Babe, and Masaki Fukuzumi

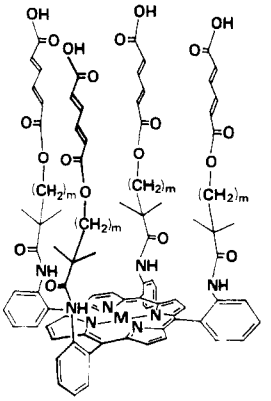
Department of Polymer Chemistry, Waseda University, Tokyo 160, Japan.
Received March 9, 1988

ABSTRACT: This paper describes the synthesis of polymerizable and amphiphilic porphyrins, 5,10,15,20-tetrakis[$\alpha,\alpha,\alpha,\alpha$-*o*-(*n*′-[((4″-carboxybutadienyl)carbonyl)oxy]-2′,2′-dimethylalkanamido)phenyl]porphyrin (**1**) and 5,10,15,20-tetrakis[$\alpha,\alpha,\alpha,\alpha$-*o*-(*n*′-[((2″-carboxypropenyl)carbonyl)oxy]-2′,2′-dimethylalkanamido)-phenyl]porphyrin (**2**), and their copolymers with 1,2-bis(2′,4′-octadecadienoyl)-*sn*-glycero-3-phosphocholine. **1**, **2**, and their (porphinato)iron derivatives have high compatibility with phospholipids and are efficiently copolymerized with the polymerizable phospholipid in a liposome state to give polymerized liposomes covalently containing the porphyrin residues. The oxygen-binding property of the copolymerized (porphinato)iron liposome is also described in comparison with that of a red blood cell.

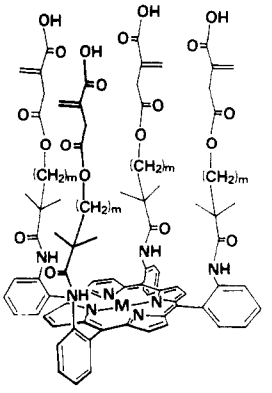
Introduction

Porphyrins and (porphinato)metals play key roles in biological and biomimetic reaction systems[1] and in molecular electronic devices.[2] In these systems much attention has been recently paid to the position and orientation of the porphyrins in matrices: (porphinato)manganese, -zinc, and -iron situated in an electron-transfer chain,[3-5] (porphinato)iron as a hemoglobin-like oxygen carrier,[6,7] and a porphyrin fixed in a polymer matrix used for photochemical hole burning.[8]

We intended to fix porphyrins and (porphinato)metals in a lipid membrane with respect to their orientation, and we preliminarily reported that polymerizable and amphiphilic porphyrins were copolymerized with a polymerizable phospholipid and fixed with a specific orientation in a bilayer membrane.[9] This paper describes the synthesis of novel porphyrin derivatives substituted with tetra($\alpha,\alpha,\alpha,\alpha$-alkyl) groups having both a polymerizable double bond and a hydrophilic (carboxylic acid) group at their top positions: 5,10,15,20-tetrakis[$\alpha,\alpha,\alpha,\alpha$-*o*-(*n*′-[((4″-carboxybutadienyl)carbonyl)oxy]-2′,2′-dimethylalkan(*n*)amido)-phenyl]porphyrins (*n* = 8, **1a**; *n* = 14, **1b**; *n* = 20, **1c**) and their (porphinato)irons (*n* = 8, **1d**; *n* = 14, **1e**; *n* = 20, **1f**) and 5,10,15,20-tetrakis[$\alpha,\alpha,\alpha,\alpha$-*o*-(*n*′-[((2″-carboxypropenyl)carbonyl)oxy]-2′,2′-dimethylalkan(*n*)amido)-phenyl]porphyrins (*n* = 8, **2a**; *n* = 14, **2b**; *n* = 20, **2c**), and their (porphinato)irons (*n* = 8, **2d**; *n* = 14, **2e**; *n* = 20, **2f**).



		m = n - 2		
		6	12	18
M	2H	1a	1b	1c
	Fe	1d	1e	1f



		m = n - 2		
		6	12	18
M	2H	2a	2b	2c
	Fe	2d	2e	2f