with a JEOL JNM-PMX 60 instrument. ^{13}C NMR spectra (100 MHz) spectra were recorded with JEOL GX-400 instrument in $CDCl_3$. Gel permeation chromatograms were obtained with Toyo Soda HLC-802 instrument with UV or refractive index detection. THF was the carrier solvent at a flow rate of 1.4 mL min^{-1} and a temperature of 40 °C. Vapor pressure osmometry (VPO) measurements were made with a Corona 117 instrument with highly sensitive thermocouples and equipment of very precise temperature control. With this instrument, molecular weight up to 20×10^4 could be measured within analytical error of 10%. Intrinsic viscosities were obtained for all polymers in THF at 40 °C by using Ubbelohde-type viscometers.

Registry No. 1, 102920-03-6; 1 (homopolymer), 102920-04-7; (1)($C_6H_5CH{=}CH_2$) (block copolymer), 112220-23-2; (1)($C_6H_5C(CH_3){=}CH_2$) (block copolymer), 112220-24-3; (1)($H_2C{=}CHC(CH_3){=}CH_2$) (block copolymer), 112220-25-4; $C_6H_5C(CH_3){=}CH_2$, 98-83-9; 4-$H_2C{=}CHC_6H_4COCl$, 1565-41-9; 4-$H_2C{=}CHC_6H_4CO_2H$, 1075-49-6; $HOCH_2C(NH_2)(CH_3)_2$, 124-68-5; 4-$HOCH_2C(CH_3)_2CH_2NHC_6H_4CH{=}CH_2$, 112220-26-5; potassium naphthalenide, 4216-48-2; lithium naphthalenide, 7308-67-0; sodium naphthalenide, 3481-12-7; cumyl potassium, 3003-91-6.

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Cholesterol-Containing Polymeric Vesicles: Syntheses, Characterization, and Separation as a Solid Powder

Iwhan Cho* and Kwang-Choon Chung

Department of Chemistry, Korea Advanced Institute of Science and Technology, Chongyangni, Seoul 131, Republic of Korea.

Received August 25, 1986

ABSTRACT: A cholesteryl amphiphilic methacrylate monomer, [[(cholesteryloxy)carbonyl]methyl][2-(methacryloyloxy)ethyl]dimethylammonium chloride (CHODAMA), was synthesized from cholesteryl chloroacetate by reacting it with 2-(dimethylamino)ethyl methacrylate, and a double alkyl chain amphiphilic methacrylate monomer, [[[[α,α'-(dihexadecyloxy)glyceryl]oxy]carbonyl]methyl][2-(methacryloyloxy)ethyl]-dimethylammonium chloride (HELDAMA), was synthesized from α,α'-(dihexadecyloxy)glycerol. The size of copolymeric vesicles prepared from CHODAMA and HELDAMA could be increased by increasing the mer content of HELDAMA in the copolymers. Poly(HELDAMA) vesicles were ruptured in 35% ethanol or by addition of surfactant Triton X-100 (0.5% aqueous solution). However, poly(CHODAMA) vesicles were found to be stable under those conditions. As the salt concentration in the vesicle-forming polymerization mixture was raised, larger vesicles resulted. The vesicles formed by homopolymers of CHODAMA were excessively stable and did not precipitate even in saturated KCl solution. When the poly(CHODAMA) vesicles prepared by polymerization in the 0.4% uranyl acetate solution were dialyzed and aged for 1 day at room temperature, the addition of acetic acid converted those vesicles to right-handed helical tubular structures which were supposed to be the result of successive fusion of vesicles. The leakage rate of entrapped [^{14}C]sucrose by poly(CHODAMA) vesicles was considerably lower than that exhibited by poly(HELDAMA) vesicles. Poly(HELDAMA) vesicles were ruptured thermally at 40 °C, whereas copolymeric vesicles containing 25% CHODAMA were stable up to 80 °C. Solid powder of poly(CHODAMA) vesicles entrapping [^{14}C]sucrose which was obtained by freeze-drying of the vesicle solution was redispersable into water and those redispersed vesicles were found to retain 90% of the [^{14}C]sucrose originally entrapped. The level of [^{14}C]sucrose-entrapping in the vesicles dropped to 43% after 4 months of standing at room temperature.

Recently, the effort to construct synthetic vesicle systems which can be employed as mimic biomembrane systems has become intense. This is based on the obvious expectation that a successful endeavor in vesicle science may lead to various applications of practical value.[1]

Formation of bilayer vesicles by a totally synthetic surfactant, didodecyldimethylammonium bromide, was first reported by T. Kunitake et al.[2] Vesicles could be formed by synthetic amphiphiles composed of one, two, or three alkyl chains (hydrocarbons or fluorocarbons) and polar head groups such as quaternary ammonium, carboxylate, sulfate, sulfonate, hydroxide, or phosphate ions.[3] The vesicles formed by synthetic dialkyl surfactant are thermodynamically unstable, undergoing successive fusion on standing. Thus, all the possible applications, especially those for which long-term stability is required such as drug carriers or models for biological membranes, become very limited. The need for increased stability and controllable permeabilities has led to the syntheses of polymeric surfactant vesicles. Regen and his co-workers reported the first syntheses of polymerized surfactant vesicles.[4] Ringsdorf's group also reported polymeric bilayer vesicles

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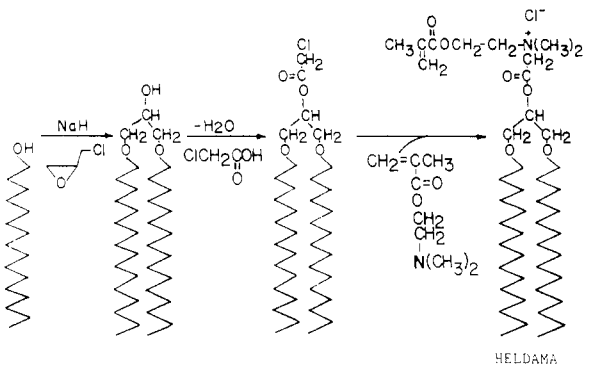
Scheme I



formed by dialkyl amphiphilic monomers containing diacetylene groups.[5] However, polymeric vesicles which retain intrinsic bilayer characteristics were first reported by Kunitake.[7] Since then vesicle-forming surfactant monomers functionalized with different polymerizable groups such as methacrylate,[4,6-9] vinyl,[10-12] diacetylene,[5,13] isocyano,[14] and thiol[15] groups have been reported. Various polymerized vesicles show different permeabilities and sizes and often possess good stability, showing shelf lives of many months. We have reported recently the synthesis of cholesterol-containing cationic methacrylate monomer (CHODAMA) and the concurrent formation of stable unibilayer microvesicles upon polymerization. In the second phase of our work we have copolymerized CHODAMA with dialkyl amphiphilic monomers in an attempt to control the thermal stability of the resulting polymeric vesicles. On these copolymer vesicles, permeability and fusion studies were performed and the results are reported in the present paper.

Taking advantage of the high stability of poly(CHODAMA) vesicles, we also have carried out lyophilization experiments in which solutions containing substrate-entrapped vesicles were freeze-dried and then redispersed into water after storage for a certain time period. Successful results of this type of experiment may be significant, when one considers certain practical applications of vesicles. The results obtained with poly(CHODAMA) vesicles are also presented in the present paper.

Results and Discussion

Syntheses of Amphiphilic Methacrylate Monomers. Cholesteryl amphiphilic methacrylate monomer, [[(cholesteryloxy)carbonyl]methyl][2-(methacryloyloxy)ethyl]-dimethylammonium chloride, coded as CHODAMA, was prepared from cholesteryl chloroacetate by reacting with 2-(dimethylamino)ethyl methacrylate, as we have reported previously.[16] CHODAMA was soluble in water, giving a viscous solution (Scheme I).

A double alkyl chain amphiphilic methacrylate monomer, [[[[α,α'-(dihexadecyloxy)glyceryl]oxy]carbonyl]-methyl][2-(methacryloyloxy)ethyl]dimethylammonium chloride, coded as HELDAMA, was prepared from α,α'-(dihexadecyloxy)glycerol according to the procedure similar to that employed for CHODAMA. Didodecyl amphiphilic methacrylate monomer, coded as DOLDAMA, was also prepared in a similar manner. DOLDAMA was readily dispersed in water at room temperature, whereas HELDAMA which was more hydrophobic than DOLDAMA could be dispersed in water only at temperatures above its phase-transition temperature (42.5 °C).

Preparation of Vesicles. CHODAMA could be polymerized in water either by free radical initiators or by UV irradiation. In spite of its bulky pendent group, the monomer polymerized readily in water, suggesting the alignment of monomer molecules in the aqueous phase. As the polymerization took place, CHODAMA changed its phase to polymeric microvesicles. It was interesting to observe that the viscosity of solution actually decreased as polymerization proceeded.

Monomeric dialkyl amphiphiles were first dispersed in water by sonication at the temperatures above their phase-transition temperatures. In this solution monomeric vesicles were formed. These monomeric vesicles were then subjected to polymerization either by irradiation with a UV lamp for 3 h or by heating the monomer solution with an initiator (potassium persulfate) at 70 °C for 12 h. Dispersion of HELDAMA in water increased its clarity by sonication, but a certain degree of turbidity developed during the polymerization reaction.

Formation of vesicles by monomeric and polymeric dialkyl amphiphiles was confirmed by electron micrographs and by entrapping experiments.

The vesicles formed by copolymers of CHODAMA and DOLDAMA or HELDAMA were prepared by UV irradiation (33 °C, 3 h) of the vesicles formed by monomeric mixtures. Comonomer vesicles were prepared by sonication at temperatures above the phase-transition temperatures of comonomer mixtures of CHODAMA and dialkyl amphiphilic monomers as a dispersion in water.

The polymer formation of vesicles was followed by the change in IR spectra. In IR spectrum of the lyophilized vesicles, the absorption peak at 1645 cm^{-1} by vinyl group disappeared completely. Monomer CHODAMA was completely soluble in water. However, solid poly(CHODAMA) vesicles obtained by drying under vacuum at room temperature, not by lyophilization, were insoluble in water.

Morphologies of Vesicles. The aggregate morphologies of polymerized vesicles were observed by electron microscopy. The formation of closed spherical poly(CHODAMA) vesicles having diameters ranging from 200 to 500 Å was confirmed by electron micrographs. Poly(CHODAMA) vesicles prepared by a water-soluble initiator or UV irradiation displayed unibilayer vesicle structures, while monomeric CHODAMA in water was observed only as randomly clustered structures even after prolonged sonication, as shown by electron micrograph.[16] It is conceivable that poly(CHODAMA) forms thermally stable vesicles by efficient packing of cholesterol groups and the packing should be further affected by tighter arrangement of the polar head groups by polymerization.[17,18]

Monomeric DOLDAMA and HELDAMA which have two alkyl chains as hydrophobic moiety formed spherical multilamellar vesicles upon sonication at temperatures above their phase-transition temperatures. The sizes of dialkyl amphiphilic vesicles were observed by electron microscopy as having diameters ranging from 300 to 1500 Å, even though the size varied depending upon the sonication conditions.

Polymeric CHODAMA vesicles were very stable, as we have reported previously. No appreciable change was observed even after 8 months of its formation. It was notable that when formed once, poly(CHODAMA) vesicles did not precipitate upon the addition of KCl even when saturated, whereas monomeric HELDAMA vesicles precipitated by the addition of a small amount of salt.[19]

As the salt concentration in monomer solution was increased, the resulting poly(CHODAMA) vesicles became larger, probably by osmotic pressure generated during the vesicle formation. Electron micrographs indicated that when the vesicles became larger than a certain size (approximately 800 Å), the vesicles exhibited a tendency to form multibilayer structures. Poly(CHODAMA) vesicles

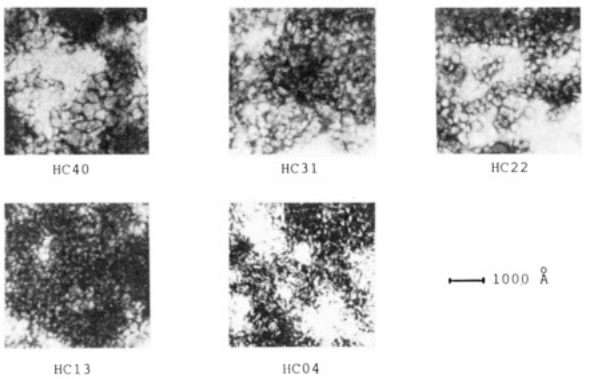


Figure 1. Electron micrographs of copolymeric vesicles of HELDAMA and CHODAMA prepared by sonication. Mole ratio of HELDAMA and CHODAMA: HC40, HELDAMA; HC31, 3:1; HC22, 2:2; HC13, 1:3; HC04, CHODAMA. Magnification, ×48000; negative staining JEM-100CX (Jeol).

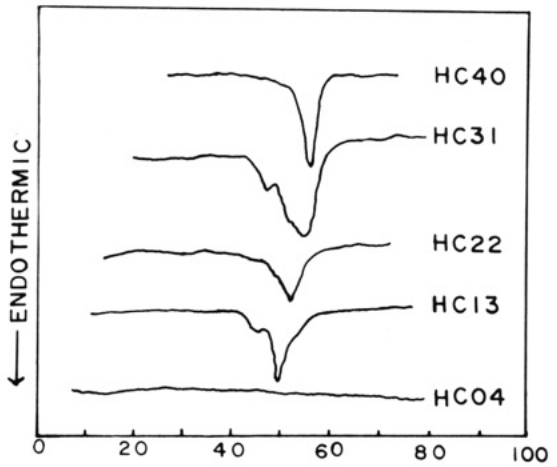


Figure 2. Phase transition curve for copolymeric bilayer vesicles: concentration, ca. 10 wt %; heating rate, 5 °C/min; Du Pont Model 910 was used. Mole ratio of HELDAMA and CHODAMA: HC40, HELDAMA; HC31, 3:1; HC22, 2:2; HC13, 1:3; HC04, CHODAMA.

prepared in 0.4% KCl solution formed multilamellar structures. When the salt concentration was higher than 0.5%, the polymers precipitated during the polymerization reaction.

The size of the bilayer vesicles formed by copolymers of CHODAMA and HELDAMA could be increased by increasing the mer content of HELDAMA in the copolymers (Figure 1). It has long been known that in biomembrane systems cholesterol incorporates into bilayer lipid membranes to a significant level, exhibiting a stabilization effect on the membranes. In the present study we have also observed that cholesterol groups of CHODAMA help stabilizing the large vesicles which poly(HELDAMA) tends to form.

Phase Transitions. Phase transitions of monomeric aggregates of CHODAMA and HELDAMA in water were determined to be at 25.0 and 42.5 °C. The polymeric vesicles exhibited quite different phase-transition behaviors. Poly(CHODAMA) vesicles did not show any transition up to 100 °C, while poly(HELDAMA) vesicles exhibited its transition at 56.5 °C.

Copolymeric vesicles of CHODAMA and HELDAMA of different compositions showed that phase-transition temperatures of those copolymeric vesicles were lowered as the mer content of HELDAMA increased. In contrast to the homopoly(HELDAMA) vesicles which exhibited a sharp single phase transition, copolymeric vesicles with CHODAMA showed only a broad phase transition profile with unidentifiable minor transitions in the DSC thermograms (Figure 2).

Table I
Entrapment of [^{14}C]Sucrose by the Homo- and Copolymeric Bilayer Vesicles of CHODAMA and HELDAMA

copolymeric vesicles[a]	HC40	HC31	HC22	HC13	HC04
entrapment,[b] %	1.07	0.78	0.57	0.55	0.48

[a] Conditions of copolymeric vesicle formation; HELDAMA + CHODAMA, 8 mM; distilled water, 1 mL; [^{14}C]sucrose, 1 μCi (5 μL); sonication at 55 °C for 5 min; polymerization by UV (254 nm) for 3 h. Mole ratio of HELDAMA and CHODAMA: HC40, HELDAMA; HC31, 3:1; HC22, 2:2; HC13, 1:3; HC04, CHODAMA. [b] Gel permeation chromatography, Sephadex G-50-80; liquid scintillation counting, Beckman Model LS-3133T scintillation spectrometer.

Table II
Retention of [^{14}C]Sucrose in Polymeric Bilayer Vesicles

	retention, %[b]		
vesicles[a]	4 h	8 h	24 h
PV–HELDAMA	88.8	75.0	47.6
PV–CHODAMA	89.0	79.0	71.0

[a] The monomer was first sonicated at 55 °C for 5 min and then polymerized for 3 h by UV. [b] Retention (%) was determined after gel filtration on Sephadex G-50-80.

Entrapment. In order to demonstrate that polymeric vesicles were indeed closely sealed spheres, entrapping of [^{14}C]sucrose into the vesicles was carried out with the vesicles formed by homo- and copolymers of CHODAMA and HELDAMA. The results are summarized in Table I.

As shown in Table I, the level of [^{14}C]sucrose entrapping increased as the ratio of HELDAMA to CHODAMA in the copolymers was raised. This increase in the amount of entrapped [^{14}C]sucrose by the copolymeric vesicles could be attributed to the difference in the size of copolymeric vesicles. Increasing the portion of HELDAMA, the size of copolymeric vesicles increased as indicated above and entrapped more substrates.

In a separate experiment HELDAMA monomer was suspended in aqueous solution (0.05%) of Brilliant Green dye containing potassium persulfate. After sonication, polymerization was continued at 70 °C for 12 h. Dye-entrapped poly(HELDAMA) vesicles were pale green in color, when dialyzed at room temperature for 3 days, and the vesicles were found to have entrapped 2.9% of the dye.[20]

Leakage and Rupture. Leakage through vesicular membranes can be measured by the use of various water-soluble marker molecules. Commonly used detection methods are those based on fluorescence, enzymatic, redox, electron paramagnetic resonance, and radiochemical techniques. [^{14}C]Sucrose has been used widely for this purpose, because it does not interact with ionic membrane lipids and can be used at low ionic strength. [^{14}C]Sucrose was also chosen for the present study and leakage rates of [^{14}C]sucrose by poly(CHODAMA) and poly(HELDAMA) vesicles were determined. The results showed that poly(CHODAMA) vesicles exhibited considerably slower leakage rate than poly(HELDAMA) vesicles, indicating that cholesterol moiety of poly(CHODAMA) contributes to form tighter membranes of less permeability.[21,22]

The rupture temperatures of poly(HELDAMA) vesicles is shown in Figure 3. Poly(HELDAMA) vesicles were ruptured thermally above 40 °C, whereas copolymeric vesicles containing more than 25 mol % CHODAMA did not show any indication of rupturing even at 80 °C.

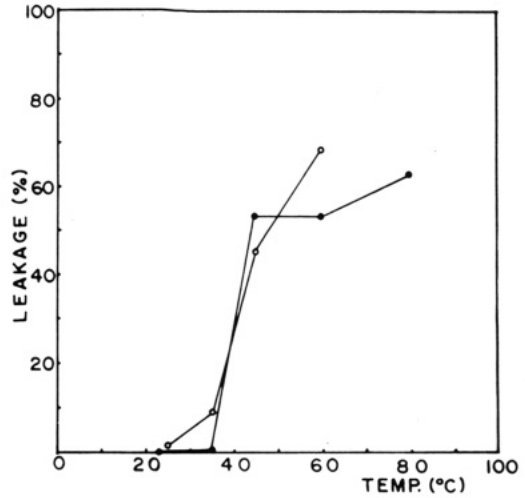


Figure 3. Leakage of [^{14}C]sucrose (○) and brilliant green dye (●) for 30 min in poly(HELDAMA) vesicles.

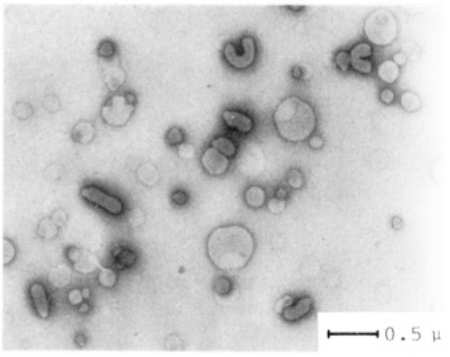


Figure 4. Electron micrograph of uranyl acetate entrapped poly(CHODAMA) vesicles prepared by radical polymerization without sonication under 0.4% uranyl acetate solution and dialyzed at room temperature for 3 days: magnification, ×14000.

Dye-entrapped poly(HELDAMA) vesicles were found to rupture in 35% ethanol, as observed by the sudden increase in absorbance at 620 nm.[8] The addition of surfactant Triton X-100 (0.5% aqueous solution) to the poly(HELDAMA) vesicle solution also induced the rupture of the vesicles. However, the addition of ethanol or surfactant Triton X-100 to the poly(CHODAMA) vesicle solution induced no such vesicle rupture.

Formation of Helical Superstructures. When CHODAMA was polymerized radically in aqueous 0.4% uranyl acetate solution, dialyzed against 2% sodium chloride solution, and aged at room temperature for certain time periods, electron micrographs revealed that uranyl acetate was entrapped in the vesicles and the vesicle size was grown to the size ranging from 3000 to 5000 Å in diameter (Figure 4).

Interesting to observe was that as the above dialyzed solution was diluted with dilute acetic acid to pH 4.0 and aged for 1 day at room temperature, electron micrographs showed the appearance of right-handed helical tubular structures (Figure 5). We assume that the formation of this giant tubular structures were the consequence of extensive fusion of vesicles.

The fusion mechanism has been the subject of many studies and it is noted that the presently investigated cholesterol-containing polymeric amphiphiles provide an interesting study system in which vesicles fuse to form giant helical tubular structures.[23] Recently, Kunitake et al. have reported the formation of so-called "helical super structures" formed by low molecular weight surfactant molecules.[23-25] The formation of helical tubular structures

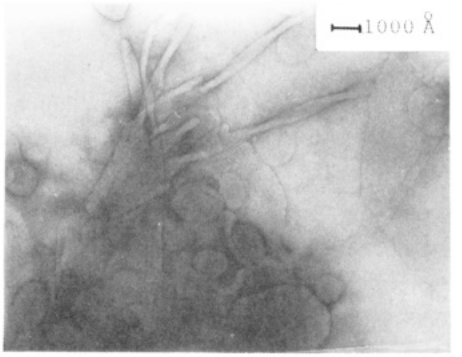


Figure 5. Electron micrograph of uranyl acetate entrapped poly(CHODAMA) helical tubular structures prepared by radical polymerization without sonication under 0.4% uranyl acetate solution, dialyzed at room temperature for 3 days, and aged at room temperature for 1 day after dilution (6 times, dilute acetic acid solution): magnification, ×35000.

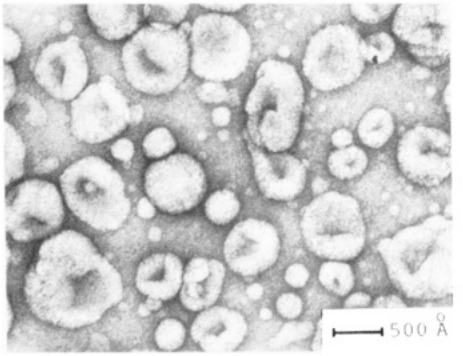


Figure 6. Electron micrograph of negatively stained poly(CHODAMA) vesicles prepared by redispersion of lyophilized poly(CHODAMA) vesicles: magnification, ×134400.

of poly(CHODAMA) may be attributed to the rigid helical packing of hydrophobic cholesterol moieties in water. It is a significant result of the present study to find that the formation of the helical tubular structures is also possible with polymeric amphiphiles such as poly(CHODAMA) and the mechanism of the formation, properties, functions, etc. of those tubular structures requires further studies.

Lyophilization and Redispersion of Vesicles. In order to explore the possibility of separating and storing the vesicles in solid powder form, taking advantage of high stability of poly(CHODAMA) vesicles, the solution of poly(CHODAMA) vesicles containing [^{14}C]sucrose was first gel-filtered to remove free [^{14}C]sucrose and then lyophilized. Lyophilized powder was stored for certain time periods and then again suspended into distilled water. Electron micrographs confirmed the regeneration of vesicles of the diameters ranging approximately between 300 and 700 Å (Figure 6).

Retaining of [^{14}C]sucrose in the internal aqueous phase of poly(CHODAMA) vesicles was confirmed by liquid scintillation counting after gel permeation chromatography of the regenerated [^{14}C]sucrose-entrapped poly(CHODAMA) vesicle solution. The results indicated that 90% of [^{14}C]sucrose originally entrapped in the poly(CHODAMA) vesicles was retained.

Upon standing in solution for 4 months, the vesicles were found to retain at least 43% of the [^{14}C]sucrose entrapped in the original vesicles (Figure 7). The lyophilized vesicle powder entrapping the substrates may be useful for practical long-term applications of vesicles.

Experimental Section

General Methods. Solvents were purified or dried by literature methods. The other reagents were commercial grade and ade-

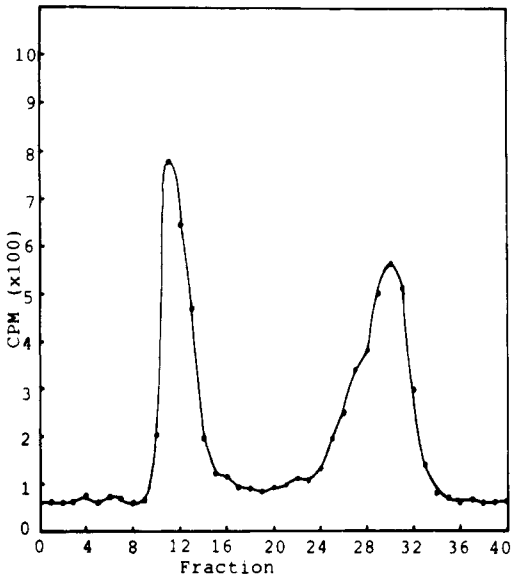


Figure 7. Column profile for the separation of [^{14}C]sucrose entrapped into the regenerated poly(CHODAMA) vesicles from free [^{14}C]sucrose on a 1.3 × 31 cm Sephadex G-50-80 column.

quately purified before use by standard methods. Authentic samples of [^{14}C]sucrose were purchased from Amersham, Inc. Triton X-100 and toluene which were used in the cocktail solution for liquid scintillation counting were liquid scintillation grades purchased from Merck, Inc.

Infrared spectra were recorded on a Perkin-Elmer Model 267 spectrometer. NMR spectra were obtained on Varian T-60A spectrometer with tetramethylsilane as an internal standard. Ultraviolet–visible spectra were recorded on Cary 17 spectrophotometer. Phase-transition temperatures of vesicles were obtained by Du Pont M 990 differential scanning calorimeter. UV reactor for the polymerization of monomers was Rayonel Srinivasan Griffin photochemical reactor.

Sonication for the vesicle formation was carried out by Branson's bath type sonicator B-52 (sonic power, 240 W). Liquid scintillation countings for the entrapment and leakage of [^{14}C]-sucrose were carried out by Beckman Model LS-3133T scintillation spectrometer.

Cholesteryl Methacryloyl Ammonium Lipid (CHODAMA). Cholesteryl chloroacetate (25 g, 54 mM) and 25 g (159 mM) of distilled 2-(dimethylamino)ethyl methacrylate were dissolved in 250 mL of dry tetrahydrofuran. The mixture was stirred for 3 days at room temperature. The turbid solution was then filtered, washed with tetrahydrofuran, and dried. The product obtained was 13.8 g (41% yield): mp 172 °C (with polymerization); ^{1}H NMR ($CDCl_3$) δ 0.8–2.4 (br m, 43 H, CH_3CHCH_2), 1.95 (s, 3 H, CCH_3), 3.75 (s, 6 H, NCH_3), 4.45 (m, 2 H, NCH_2), 4.5–4.8 (br, m, CCHOCO), 4.63 (m, 2 H, $COOCH_2$), 4.95 (s, 2 H, NCH_2CO), 5.4 (m, 1 H, CCH=C), 5.65 (s, 1 H, CH=C), 6.1 (s, 1 H, CH=C); IR (KBr) $\nu_{C=O}$ 1745, $\nu_{C=C}$ 1645 cm^{-1}. Anal. Calcd for $C_{37}H_{62}O_4NCl$: C, 71.64; H, 10.07; N, 2.26. Found: C, 68.31; H, 10.14; N, 2.08.

α,α′-(Dihexadecyloxy)glycerol. α,α′-(Dihexadecyloxy)-glycerol was prepared according to the procedures of Okahata et al.[26] Epichlorohydrin (9.2 g, 100 mM) was added with stirring to excess hexadecyl alcohol (210 g, ca. 10 times excess) at 60 °C in the presence of 4.4 g of 60% NaH mineral oil dispersion, and the mixture was then stirred for 6 h. The crude product was extracted with ether, and the extracted solution was filtered and washed with water. After the solvent stripping, the product was recrystallized 3 times from acetone. The product obtained was 41 g (87.7% yield): mp 58–59 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.28 (br s, 56 H, CH_2), 2.5 (br s, 1 H, OH), 3.5 (m, 8 H, OCH_2), 3.85 (t, 1 H, OCH); IR (KBr) ν_{OH} 3495, ν_{COC} 1120 cm^{-1}.

α,α′-(Dihexadecyloxy)glyceryl Chloroacetate. α,α′-(Dihexadecyloxy)glycerol (27 g, 50 mM) and 7.1 (75 mM) of chloroacetic acid were dissolved in 200 mL of toluene and heated to reflux with a Dean and Stark set for 36 h. After 0.8 mL of water was distilled off, excess chloroacetic acid was removed by washing 3 times with water, 1 time with 10% $NaHCO_3$ solution, and then again 3 times with water. The solvent was evaporated, and the residue was crystallized from chloroform giving final product, 27.8 g (90% yield): mp 49.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.25 (br s, 56 H, CH_2), 3.5 (t, 4 H, OCH_2), 3.65 (d, 4 H, J = 5 Hz, $OCCH_2O$), 4.15 (s, 2 H, $ClCH_2CO$), 5.25 (t, 1 H, COOCH); IR (KBr) $\nu_{C=O}$ 1750 cm^{-1}. Anal. Calcd for $C_{37}H_{73}O_4Cl$: C, 71.97; H, 11.91. Found: C, 71.29; H, 12.01.

Dihexadecyl Methacryloyl Ammonium Lipid (HELDA-MA). α,α′-(Dihexadecyloxy)glyceryl choloroacetate (3.1 g, 5 mM), 3.15 g (20 mM) of 2-(dimethylamino)ethyl methacrylate, and 6 mg of *p*-methoxyphenol (inhibitor) were dissolved in 50 mL of dry acetone. The reaction mixture was slowly heated up to reflux condition. The precipitated product was then filtered and washed with ether. Recrystallization from acetone gave 3.5 g (91% yield) of the desired product: mp 165 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6 H, CH_3), 1.26 (br s, 56 H, CH_2), 1.95 (s, 3 H, CCH_3), 3.4 (t, 4 H, OCH_2), 3.5 (d, 4 H, J = 5 Hz, $OCHCH_2O$), 3.75 (s, 6 H, NCH_3), 4.45 (m, 2 H, NCH_2), 4.63 (m, 2 H, $COOCH_2$), 4.95 (br s, 2 H, NCH_2CO), 5.2 (t, 1 H, COOCH), 5.65 (s, 1 H, CH=C), 6.1 (s, 1 H, CH=C); IR (KBr) $\nu_{C=O}$ 1760, 1730, $\nu_{C=C}$ 1645 cm^{-1}. Anal. Calcd for $C_{45}H_{88}O_6NCl$: C, 69.77; H, 11.45; N, 1.81. Found: C, 69.05; H, 11.52; N, 1.78.

α,α′-(Didodecyloxy)glycerol. α,α′-(Didodecyloxy)glycerol was prepared according to the procedures of Okahata et al.[26] Epichlorohydrin (4.7 g, 50 mM) was added with stirring to excess dodecyl alcohol (100 g, ca. 10 times excess) at 60 °C in the presence of 1 equiv of NaH (2.2 g, 60% in mineral oil) dispersion, and the mixture was kept stirred for the additional 6 h. The crude product was extracted with ether and isolated by distillation. The product obtained was 19.2 g (84% yield): mp 41–42 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.28 (br s, 40 H, CH_2), 2.5 (br s, 1 H, OH), 3.5 (m, 8 H, OCH_2), 3.85 (t, 1 H, OCH); IR (KBr) ν_{OH} 3490, ν_{COC} 1120 cm^{-1}.

α,α′-(Didodecyloxy)glyceryl Chloroacetate. α,α′-(Didodecyloxy)glycerol (5.0 g, 11.5 mM) and 2.2 g (23 mM) of chloroacetic acid in 50 mL of toluene were heated to reflux with a Dean and Stark set for 36 h. After 0.22 g of water was distilled off, the excess chloroacetic acid was removed by washing 3 times with water, 1 time with 10% $NaHCO_3$ solution, and then again 3 times with water. The solvent was evaporated, and the residue was recrystallized from acetone. The product obtained was 5.3 g (86% yield): mp 29.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.25 (br s, 40 H, CH_2), 3.5 (t, 4 H, OCH_2), 3.65 (d, 4 H, J = 5 Hz, $OCHCH_2O$), 4.15 (s, 2 H, $ClCH_2CO$), 5.25 (t, 1 H, COOCH); IR (KBr) $\nu_{C=O}$ 1750 cm^{-1}. Anal. Calcd for $C_{29}H_{57}O_4Cl$: C, 68.94; H, 11.37. Found: C, 68.28; H, 11.48.

Didodecyl Methacryloyl Ammonium Lipid (DOLDAMA). α,α′-(Didodecyloxy)glyceryl chloroacetate (3.74 g, 7.4 mM), 2.33 g (14.8 mM) of 2-(dimethylamino)ethyl methacrylate, and 5 mg of *p*-methoxyphenol (inhibitor) were dissolved in 50 mL of dry acetone. The reaction mixture was heated to reflux for 12 h. At room temperature the precipitated product was filtered and washed with ether and the product was recrystallized 3 times from acetone. The product obtained was 3.48 g (71% yield): mp 150 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6 H, CH_3), 1.26 (br s, 40 H, CH_2), 1.95 (s, 3 H, CCH_3), 3.4 (t, 4 H, OCH_2), 3.5 (d, 4 H, J = 5 Hz, $OCHCH_2O$), 3.75 (s, 6 H, NCH_3), 4.45 (m, 2 H, NCH_2), 4.63 (m, 2 H, $COOCH_2$), 4.95 (br s, 2 H, NCH_2CO), 5.2 (t, 1 H, COOCH), 5.65 (s, 1 H, CH=C), 6.1 (s, 1 H, CH=C); IR (KBr) $\nu_{C=O}$ 1760, 1730, $\nu_{C=C}$ 1645 cm^{-1}. Anal. Calcd for $C_{37}H_{72}O_6NCl$: C, 68.09; H, 10.95; N, 2.11. Found: C, 67.19; H, 11.02; N, 1.98.

Formation of a Monomeric Vesicle. A representative procedure is as follows: an amphiphilic monomer (8 mM) was mixed with water. The resulting turbid solution (0.5 wt %) was flushed with nitrogen and then was placed in a bath-type sonicator at 55 °C, and the sample was irradiated at a power level of 240 W. After 5 min of sonication, the sample solution was removed from the bath for further experiments.

Formation of Polymeric Vesicles. Cholesterol-containing polymeric bilayer vesicles were prepared by stirring the solution of 0.05 g (0.08 mM) of CHODAMA and 1 mg of water-soluble free radical initiator (potassium persulfate) in 10 mL of distilled water at 70 °C for 12 h. Poly(CHODAMA) vesicles were also prepared by UV irradiation without initiator at 25 °C for 3 h. In the case of dialkyl amphiphilic vesicles, the monomer vesicles were first

prepared by sonication of DOLDAMA or HELDAMA solutions (typically 53–62 mg of monomer in 10 mL of H_2O) at 55 °C for 5 min with Branson bath-type sonicator B-52 (sonic power, 240 W). Polymerization was then carried out either by irradiation with a UV lamp for 3 h or by heating with an initiator (potassium persulfate) at 70 °C for 12 h.

Formation of Copolymeric Vesicles. Copolymeric vesicles of CHODAMA and DOLDAMA or HELDAMA were prepared by UV irradiation (33 °C, 3 h) of comonomeric vesicles which were formed prior to polymerization. Monomeric mixtures of CHODAMA/HELDAMA in ratios of 0/4–4/0 (total, 8 mM) were dispersed by the vortex mixer in water at 55 °C for 30 min. These turbid comonomer solutions (ca. 0.5 wt %) degassed previously under nitrogen were sonicated in a bath-type sonicator at 55 °C for 5 min.

Electron Microscopy. The aggregate morphologies of polymeric vesicles prepared as describes above were observed by negative-staining transmission electron microscopy. Samples for electron microscopy were prepared by mixing equal volumes of the polymeric vesicle solution (about 0.5%, 8 mM) with a 2% uranyl acetate solution. One drop of the resulting solution was then placed on the carbon-coated copper grid, followed by blotting and drying. In this wasy the morphology of aqueous aggregates could be fixed by staining agents. Monomeric HELDAMA vesicles were prepared for electron microscopy by sonication (55 °C, 5 min) of the HELDAMA-dispersed solution in the presence of 2% aqueous uranyl acetate. Electron micrography was performed on a JEOL JEM-100X electron microscope. The accelerating voltage was 80 kV.

Stability Measurement of Polymeric Vesicles. Any change of polymeric vesicles upon prolonged standing at room temperature was observed by taking electron micrographs of the vesicles at certain time intervals. By observing the change taking place when heated to 90 °C in a certain time period, the thermal stability of the vesicles was determined. The precipitation of polymeric CHODAMA vesicles upon the addition of KCl was observed to determine the stability against salt. The stability of polymeric CHODAMA vesicles at the different salt concentration (KCl) ranging from 0.05% to 0.5% was examined by observing the tendency of precipitation and by taking the electron micrographs. Also the effect of addition of ethanol or Triton X-100 to the monomeric and polymeric vesicle solution was observed by determining the turbidity at 400 nm by Spectronic 20 spectrophotometer.

Differential Scanning Calorimeter. One drop of each of aqueous solutions of the amphiphilic monomeric or polymeric vesicle solution (ca. 0.5–2.0 wt %) was sealed in an aluminium sample pan and the measurements were performed with a differential scanning calorimeter Du Pont M 990. The heating rate was 5 °C/min.

Entrapment and Leakage Test with [^{14}C]Sucrose. [^{14}C]-Sucrose was entrapped in the vesicles derived from CHODAMA and HELDAMA by the procedures similar to those described in the section for the vesicle preparation. In this case, however, 5–6.3 mg (8 μM) of monomer was suspended in 1.0 mL of distilled water containing 1 μCi (5 μL) of [^{14}C]sucrose and sonicated at 55 °C for 5 min. After degassing under nitrogen, polymerization by UV irradiation was carried out. In the case of free radical polymerization, 0.1 mg of potassium persulfate, [^{14}C]sucrose, and monomer were mixed and polymerized at 70 °C for 12 h. The sucrose which was not entrapped was removed by gel filtration on Sephadex G-50-80; 1.25-mL fractions were eluted every 1.5 min. Typically, 1 mL of the sample solution was added to a wet 31 × 1.3 cm column and then eluted with distilled water, and 1.25-mL fraction was collected in each test tube of fraction collector.

Vesicles recovered in the void volume of the column were immediately placed in presoaked seamless cellulose bags and dialyzed against a large volume of distilled water for a certain time period in a temperature-regulated water bath (25 ± 0.5 °C). After the dialysis against the water at 25 °C for 4 h, leakage of [^{14}C]sucrose in vitro from the polymeric vesicles was measured by a liquid scintillation counter: 0.4 mL of vesicles was sampled at certain time intervals for 30 min. Aliquots (0.4 mL) of the samples to be measured were pipetted into 20-mL screw-cap glass vials. Five milliliters of cocktail solution for scintillation counting (toluene, 1 L; Triton X-100, 500 mL; PPO, 4 g; POPOP, 50 mg) was added, and the vials were mixed thoroughly with a vortex mixer before counting in a Beckman Model LS-3133T scintillation spectrometer. Column samples were counted for 2 min, and dialysis samples were counted for 10 min to obtain good stable values.

Entrapment and Leakage Test with Brilliant Green Dye. HELDAMA (155 mg, 0.2 mM) was suspended in 25 mL of 0.05% aqueous Brilliant Green dye solution containing 3 mg of potassium persulfate, followed by sonication at 55 °C for 5 min. Polymerization of HELDAMA vesicles containing dye was carried out at 70 °C for 12 h. Polymerized HELDAMA vesicles were placed in a dialysis bag and dialyzed at room temperature for 3 days against a large volume of distilled water. Dye-entrapped poly(HELDAMA) vesicles were pale green in color.

One milliliter of each of these vesicle solution was placed in a dialysis bag and then was placed in 50 mL of distilled water. Dialysis was then continued for certain time period at a certain temperature. During the dialysis the water was replaced with freshly distilled water at certain time intervals maintaining the same temperature. The aqueous solution taken out was then placed in a 100-mL flask and was concentrated, followed by the addition of a proper volume of distilled water to adjust to total volume of solution. The absorbances of these leaked dye solutions were determined on a Cary 17 spectrophotometer at 620 nm.

Redispersion of Poly(CHODAMA) Vesicles. CHODAMA (5 mg, 8 μM) was dissolved in 1.0 mL of distilled water containing 1 μCi (5 μL) of [^{14}C]sucrose and was irradiated at 33 °C for 3 h. Sucrose which was not entrapped was removed by gel filtration on Sephadex G-50-80, and then 2 mL of the vesicle solution was diluted 10 times. Diluted poly(CHODAMA) vesicle solution (20 mL) placed into the 100 mL flask was frozen rapidly in the dry ice–acetone bath and then lyophilized. Lyophilized powder was then again suspended into 2 mL of distilled water. Redispersion of vesicles was confirmed by electron microscopy. The existence of entrapment of [^{14}C]sucrose was confirmed by liquid scintillation counting after gel filtration.

Acknowledgment. We are grateful to Dr. N. S. Choi, Dr. J. K. Yeo, and S. A. Song (Lucky Central Research Institute) for the use of their electron microscope.

Registry No. CHODAMA, 93085-28-0; CHODAMA (homopolymer), 93110-94-2; HELDAMA, 111793-60-3; HELDAMA (homopolymer), 111793-61-4; (CHODAMA)(HELDAMA) (copolymer), 111793-62-5; Triton X-100, 9002-93-1; cholesteryl chloroacetate, 3464-50-4; 2-(dimethylamino)ethyl methacrylate, 2867-47-2; epichlorohydrin, 106-89-8; hexadecyl alcohol, 36653-82-4; (dihexadecyloxy)glycerol, 14690-01-8; chloroacetic acid, 79-11-8; α,α'-(dihexadecyloxy)glycerol chloroacetate, 111770-04-8; dodecyl alcohol, 112-53-8; α,α'-(didodecyloxy)glycerol, 29419-17-8; α,α'-(didodecyloxy)glyceryl chloroacetate, 111770-05-9; sucrose, 57-50-1; brilliant green, 633-03-4; ethanol, 64-17-5.

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Chirality of Polyvinyl Compounds. 6.[1] Unusual Influences of the Comonomer Structures on the Chiroptical Properties of Optically Active Vinyl Copolymers with Chirality Arising from Configurational Relationships in the Main Chain

Günter Wulff* and Pradeep K. Dhal

Institute of Organic Chemistry and Macromolecular Chemistry, University of Düsseldorf, 4000 Düsseldorf, F.R.G. *Received July 6, 1987*

ABSTRACT: In contrast to appreciable negative optical rotations shown by most of the vinyl copolymers of 3,4-*O*-cyclohexylidene-D-mannitol 1,2:5,6-bis-*O*-[(4-vinylphenyl)boronate] (1) after removal of the D-mannitol template, copolymerizations of this monomer with styrene derivatives bearing functional groups in conjugation with the phenyl ring, viz., 4-cyano-, 4-formyl-, and 4-(methoxycarbonyl)styrene, yielded copolymers, which after removal of the D-mannitol template showed positive optical rotations. Copolymers obtained by using comonomers bearing the modified versions of these functional groups, viz., 4-(aminomethyl)- and 4-(hydroxymethyl)styrene, exhibited negative optical rotations. Interestingly, upon transforming the cyano, formyl, and methoxycarbonyl groups of the copolymers to the corresponding aminomethyl and hydroxymethyl groups by polymer analogue reactions, the formerly positively rotating polymers showed negative optical rotations. With the help of chiroptical techniques, particularly circular dichroism (CD), it has become possible to elucidate the unique role of the comonomer structures in this unusual behavior of the copolymers. While the absolute configuration of the distyryl diads generated from monomer 1 remains the same with all comonomers, in the case of the comonomers with conjugated chromophoric systems strong Cotton effects are induced, thus generating positively rotating polymers. These Cotton effects arise from asymmetric conformations of the atactic comonomeric part due to perturbation by chiral distyryl diads.

Introduction

Following a symmetry-based theoretical consideration of the possibility of the existence of optical activity in vinyl polymers due to chirality arising from configurational relationships in the main chain (so called main chain chirality),[2,3,3a] we have been developing synthetic methods to realize such structures. Toward this end, a polymerizable vinyl monomer linked to an appropriate chiral template (1) was synthesized and subsequently copolymerized by free radical initiation with another vinyl monomer. The resulting copolymer, after complete removal of the template molecules, showed strong negative optical rotations.[4] Mechanistic studies showed[1] that the polymerization proceeds through an asymmetric cyclopolymerization. The optical activity of the copolymer stems from the formation of chiral distyryl diads along the main chain. They are separated from one another by the comonomer units, but the chirality of the polymer is independent of the configuration at these centers. The absolute configuration of the asymmetric diad building block has been established to be *S,S* by synthesis of suitable well-defined oligomeric model compounds.[1]

In order to explore the potentialities of this new interesting copolymer system further, we have copolymerized the template monomer 1 with numerous vinyl comonomers. The resulting copolymers for the most part showed negative optical rotations with varying magnitudes depending on the structures of the comonomers and on the copolymer compositions. However, we encountered a startling situation when using 4-nitro- and 4-cyanostyrenes as comonomers, since we obtained optically active copolymers showing appreciable positive optical rotations.[1] We had no immediate explanation for this unusual observation, which apparently questioned the general validity of the proposed mechanism of the asymmetric induction and the absolute stereochemistry of the polymer chain. Therefore, it was an important task to pursue a detailed investigation for elucidating the role of these comonomers in the observed polymer chirality. Polymers showing positive optical rotations were transformed by analogous reactions to copolymers which could also be prepared by polymerizing preformed monomers bearing identical functionalities. Their chiroptical properties could then be compared. This enabled us to substantiate further the validity of the proposed mechanism of the asymmetric copolymerization. The results of this study are described in the present paper.

Results and Discussion

Synthesis and Characterization of the Polymers.

0024-9297/88/2221-0571$01.50/0