which constitutes a chemically most exciting prospect.

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Supplementary Material Available: Tables of observed and calculated structure factor amplitudes for **1** and **2** (13 pages). Ordering information is given on any current masthead page.

Polymerized Phosphatidylcholine Vesicles. Synthesis and Characterization [1]

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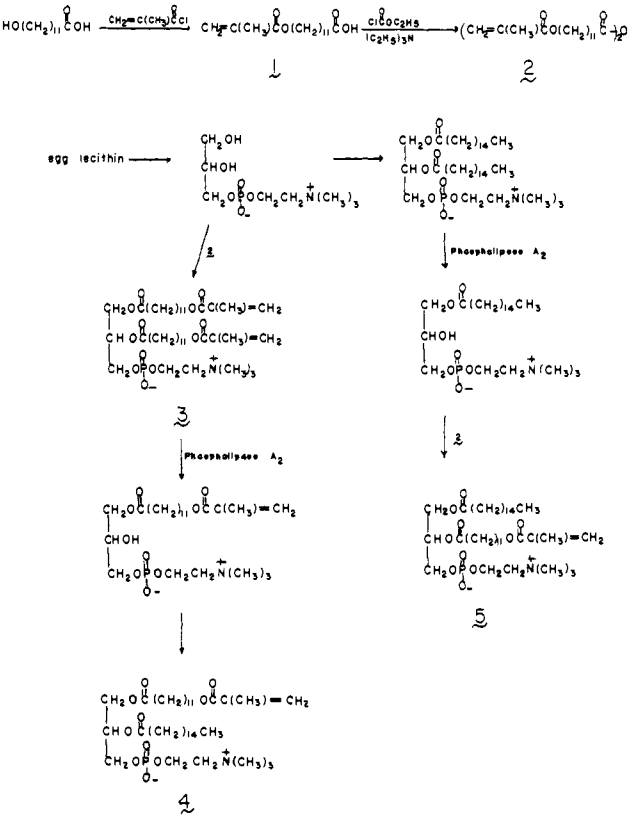
Abstract: The synthesis and characterization of photopolymerized vesicles derived from bis[12-(methacryloyloxy)dodecanoyl]-L-α-phosphatidylcholine (**3**) 1-[12-(methacryloyloxy)dodecanoyl]-2-palmitoyl-L-α-phosphatidylcholine (**4**), and 1-palmitoyl-2-[12-(methacryloyloxy)dodecanoyl]-L-α-phosphatidylcholine (**5**) are described. Ultrasonic irradiation of **3**, **4**, **5**, 20% **3** + 80% **4**, and 20% **3** + 80% **5** in water at 50 °C yields opalescent to optically clear dispersions. Electron microscopy, entrapment of [^{14}C]sucrose, and permeability measurements provide strong evidence for closed multilamellar vesicles having diameters ranging between 350 and 1400 Å. Fourier transform ^{1}H NMR spectra of the aqueous dispersions as well as IR spectra of chloroform extracts establish that no significant lipid decomposition occurs during vesicle preparation. Direct UV irradiation (254 nm) produces polymerized analogues of similar size and shape which (1) entrap [^{14}C]sucrose, (2) show reduced permeability, and (3) exhibit enhanced stability.

Introduction

Phospholipid bilayer vesicles are receiving intense interest as models for biological membranes, devices for solar energy conversion, and carriers of drugs.[3-10] Because these aggregates are unstable, having relatively short shelf lives, their use in mechanistic studies and practical applications is seriously limited.[3]

We have recently introduced the concept of polymerized vesicles and have suggested that phosphatidylcholine derivatives might constitute an important new class of materials.[11] Not only would they be expected to exhibit greater stability than liposomes (vesicles formed from naturally occurring phospholipids) but they should also retain many important properties of vesicles, e.g., the ability to (1) promote the separation of charged photoproducts and (2) entrap and slowly release drugs. Moreover, because of the likelihood of increased stability toward biological substances, such vesicles could be used as unique probes in mechanistic studies involving lipid–protein and vesicle–cell interactions. For these reasons and for the purpose of expanding the concept of the polymerized vesicles, we have set out to synthesize phosphatidylcholine analogues. The following report details our efforts.[12-14]

Scheme I



(1) This investigation was supported by PHS Grant No. CA28891-01, awarded by the National Cancer Institute, DHHS, and the National Science Foundation (Grant CHE-77-28366).

(2) On leave from the Central Institute of Organic Chemistry, Academy of Sciences of the GDR.

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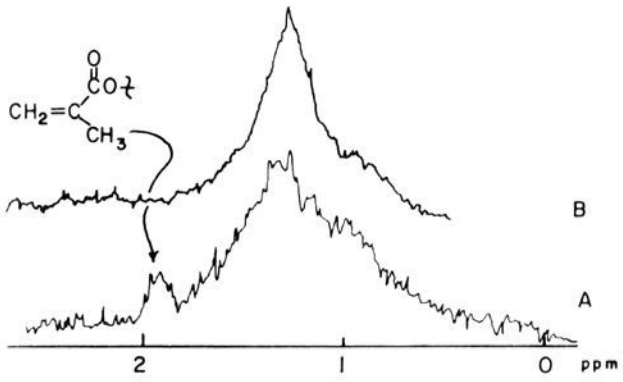

Figure 1. Fourier transform ^{1}H NMR spectra of nonpolymerized (A) and polymerized (B) vesicles of **5** recorded in D_2O at 32 °C relative to external 3-(trimethylsilyl)propionate (500 scans).

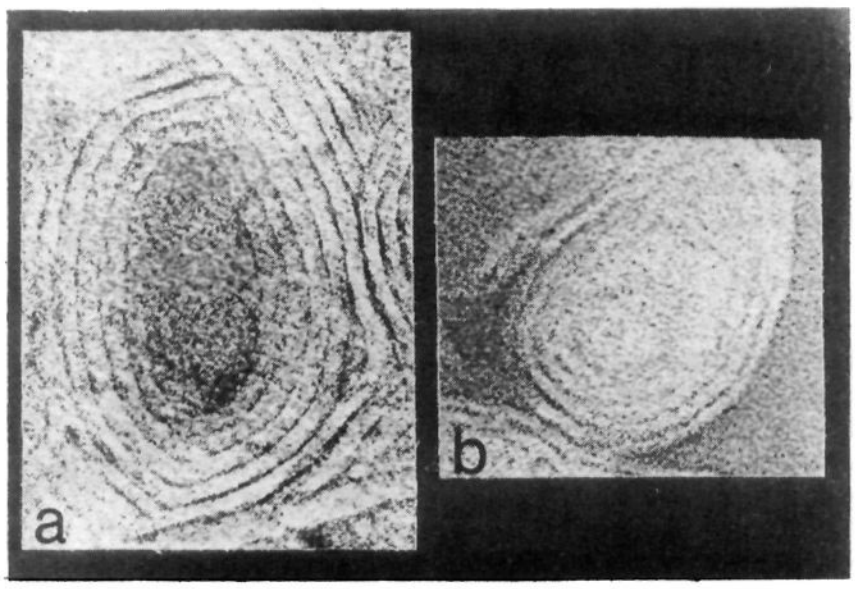

Figure 2. Electron micrographs of nonpolymerized (a) and polymerized (b) vesicles of **5** prepared in the presence of 2% aqueous uranyl acetate and phosphomolybdic acid (neutralized with NaOH) staining agents, respectively.

Results and Discussion

Lipid Synthesis. Lipids **3–5** were chosen as the target molecules from which polymerized vesicles would be constructed (Scheme I). The polymerizable methacrylate moiety was specifically positioned at the terminus of the aliphatic chain(s) in order to reduce the possibility of two or more vesicles grafting together and also to minimize changes in surface properties upon polymerization. The general synthetic approach outlined in Scheme I is based on methods previously described in the literature.[15,16] Esterification of 12-hydroxydodecanoic acid with methacryloyl chloride followed by anhydride formation yielded 12-(methacryloyloxy)dodecanoic anhydride (**2**).[17] Acylation of egg lecithin derived L-α-glycerophosphorylcholine with **2** produced bis[12-(methacryloyl)oxydodecanoyl]-L-α-phosphatidylcholine(**3**).[18,19]

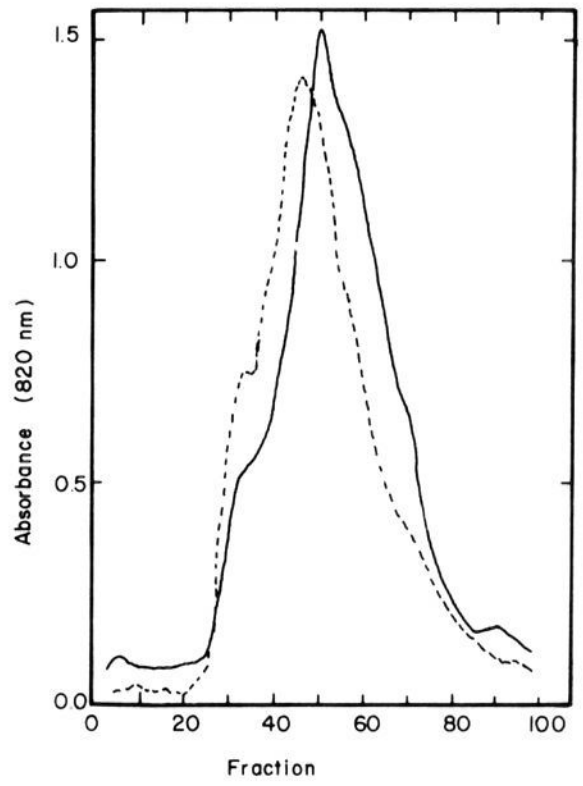

Figure 3. Gel filtration of **4** (0.006 mM) on cross-linked Sepharose 2B before (——) and after (- - -) polymerization. The mass balance for phosphorus (directly proportional to absorbance) was 92% and 91%, respectively. Elution was carried out with aqueous NaN_3 (0.02%), where 0.23-mL fractions were collected every minute. The void volume of the column was contained in fractions 1–32. Reproducibility was ±2 fractions.

Enzymatic hydrolysis of **3** with phospholipase A_2 in the form of crude rattle snake venom (*Crotalus adamanteus*) followed by acylation with palmitoyl anhydride afforded 1-[12-(methacryloyloxy)dodecanoyl]-2-palmitoyl-L-α-phosphatidylcholine (4). In order to confirm that no significant acyl migration from C-1 to C-2 occurred during the acylation of the 2-OH group, **4** was hydrolyzed with phospholipase A_2.[20] The ^{1}H NMR spectrum and TLC of the recovered fatty acid indicated that only palmitic acid was released from the phospholipid. Similar methods were used to convert dipalmitoyl-L-α-phosphatidylcholine into 1-palmitoyl-2-[12-(methacryloyloxy)dodecanoyl]-L-α-phosphatidylcholine (**5**).

Vesicle Formation. Opalescent to optically clear aqueous dispersions were obtained for vesicles prepared from lipids **3–5** by procedures described in the Experimental Section. Fourier transform ^{1}H NMR spectroscopy in all cases showed the presence of the intact vinylidene group. A representative spectrum is shown in Figure 1A. Electron micrographs taken of a preparation derived from **5** confirmed the existence of closed multilamellar vesicles having diameters ranging between 350 and 1400 Å (Figure 2a).[12] The apparent thickness of the bilayer is estimated to be approximately 100 Å. In order to ensure that no significant lipid decomposition occurred under the sonication conditions used, an aqueous preparation of **5** was extracted with chloroform; recovery of the lipid was quantitative and the IR spectrum remained unchanged.

Vesicle Polymerization. Polymerization of vesicles was effectively carried out by direct UV irradiation (254 nm).[21,22] The extent of polymerization was monitored by following the disappearance of the allyl protons in the Fourier transform ^{1}H NMR spectrum. Normally 1–3 h was required for complete reaction. Interestingly, vesicles derived from **4** polymerized more slowly than those made from **5**. Figure 1B shows a typical spectrum after polymerization. The turbidity of all preparations, except for that

(12) A preliminary account of this work has been reported recently: Regen, S. L.; Singh, A.; Oehme, G.; Singh, M. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 131.

(13) Chakrabarti and Khorana have previously synthesized phosphtidylcholines bearing a variety of photosensitive groups and have shown that UV irradiation induces intermolecular coupling within resulting vesicles. While detailed characterization of the *aqueous suspensions after photolysis* was not carried out, it is conceivable that certain of these vesicles may have been polymerized: Chakrabarti, P.; Khorana, H. G. *Biochemistry* **1975**, *14*, 5021.

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(15) Gupta, C. M.; Radhakrishnan, R.; Khorana, H. G., *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4315.

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(18) Similar yields were obtained when a $CdCl_2$ complex of L-α-glycerophosphorylcholine was used.

(19) Procedures used for hydrolysis of the lecithins were similar to those previously described in the literature: Chadha, J. S. *Chem. Phys. Lipids* **1970**, *4*, 104. Brockerhoff, H.; Yurkowski, M. *Can. J. Biochem.* **1965**, *43*, 1777.

(20) Hydrolysis procedures were similar to those previously reported.[13]

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(22) Naegele, D.; Ringsdorf, H. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 2821.

Table I. Retention of [^{14}C]Sucrose[a]

vesicle[b]	dialysis medium	treatment[c]	retention, % 4 h	8 h	24 h
5	water	none	29	16	
5 (P)	water	none	65	53	37
80% 5 + 20% 3 (P)	water	none	78	65	56
4	water	none	63	50	31
4 (P)	water	none	62	54	43
80% 4 + 20% 3 (P)	water	none	73	64	55
3	water	none	40	20	8
3 (P)	water	none	72	59	53
5	0.154 M NaCl	none	65	54	31
5 (P)	0.154 M NaCl	none	80	61	54
5	0.154 M NaCl	50 °C for 0.5 h	25		13
5 (P)	0.154 M NaCl	50 °C for 0.5 h	42	36	34
5	0.154 M NaCl	sonicated at 140 W for 0.5 h at 50 °C	15	14	10
5 (P)	0.154 M NaCl	sonicated at 140 W for 0.5 h at 50 °C	40	28	24
5	20% (v/v) ethanol	diluted to 20% (v/v) ethanol	11	8	4
5 (P)	20% (v/v) ethanol	diluted to 20% (v/v) ethanol	49	19	10

[a] Retention in dialysis bag at 23 °C. [b] All vesicles were prepared in either pure water or 0.154 M NaCl; P denotes polymerized form. [c] Treatment of vesicle sample after gel filtration and before dialysis.

of pure **3**, decreased slightly upon polymerization; with **3**, the apparent turbidity slightly increased. Also, in contrast to polymerized vesicles of **4** and **5** and copolymers of **3** and **4** and **3** and **5**, vesicles of **3**, after photolysis, did not produce an observable ^{1}H NMR spectrum. This is presumably due to line broadening caused by cross-linking and reduced mobility of the polymer chains.[23]

Attempted extraction of polymerized vesicles of **5** with chloroform failed to remove any lipid monomer, oligomer, or polymer present in the aqueous phase. Removal of water under reduced pressure followed by IR analysis of the residue (Nujol) indicated the complete disappearance of the monomer. Principal changes in the IR spectrum were the disappearance of the band at 1640 cm^{-1} ($\nu_{C=C}$), a shift of the band at 1720 cm^{-1} ($\nu_{C=O}$) to 1732 cm^{-1}, and a shift of the bands at 1300 and 1340 cm^{-1} to 1260 and 1280 cm^{-1}.[22] Electron micrographs further revealed that polymerized vesicles of **5** retain their spherical shape and approximate size (Figure 2b).[12] They also suggest that the basic bilayer structure is maintained but that a slight longitudinal contraction has taken place. Gel filtrations performed before and after UV irradiation confirmed that the size distribution of the vesicles was not significantly altered by polymerization (Figure 3).

Substrate Entrapment and Leakage. In order to demonstrate that polymerized and nonpolymerized vesicles of **3**–**5** were sealed, [^{14}C]sucrose was entrapped in the aqueous interior and its leakage rate across the vesicle membrane measured under a variety of conditions. Figure 4 shows a typical gel filtration separation of free [^{14}C]sucrose and [^{14}C]sucrose entrapped in polymerized vesicles of **5**, using a Sephadex G-50 column. Simultaneous analysis of phosphorus content and radioactivity clearly indicated the entrapment of sucrose.[24] Entrapment in most cases was ca. 0.5% and appeared unaffected by polymerization. Table I summarizes dialysis data obtained for a variety of vesicles derived from **3**–**5** under a variety of conditions. Principal conclusions that can be drawn from these results are (1) all vesicles are sealed, (2) polymerization decreases the leakage rate in pure water, 0.154 M NaCl, and 20% ethanol, (3) copolymerization with cross-linking agent **3** further reduces the leakage rate, and (4) polymerized vesicles have greater retention of [^{14}C]sucrose when subjected to heating (50 °C) and heating with sonication than do nonpolymerized vesicles.

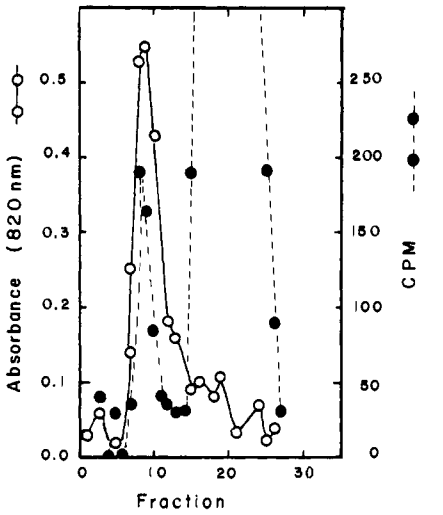

Figure 4. Gel filtration separation of free [^{14}C]sucrose and [^{14}C]sucrose entrapped in vesicles of **5**. Elution was made with distilled water, where 1.0-mL fractions were collected every minute. Phosphorus and sucrose contents directly proportional to absorbance and cpm, respectively.

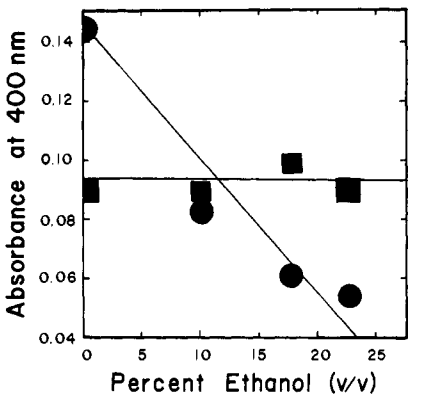

Figure 5. Plot of absorbance at 400 nm as a function of percent ethanol (v/v) for polymerized (■) and nonpolymerized (●) vesicles of **5**, where absorbandce = (observed absorbance) × (volume of aqueous vesicle dispersion + volume of ethanol added)/(volume of aqueous vesicle dispersion).

Vesicle Stability. Improved vesicle stability through polymerization was demonstrated by several means; results for **5** are representative. First, while vesicles derived from **5** precipitated

(23) James T. L. "Nuclear Magnetic Resonance in Biochemistry"; Academic Press: New York, 1975; p 322.

(24) The method of phosphorus analysis used was a modification of the method described by Ames and Dubin.[25] Specifically, ashing with $Mg(NO_3)_2 \cdot 6H_2O$ gave better reproducibility than digestion with concentrated sulfuric acid and hydrogen peroxide.[26] In control experiments, polymerized vesicles were found to have the same phosphorus content per gram as nonpolymerized vesicles.

(25) Ames, B. N.; Dubin, D. T. *J. Biol. Chem.* **1960**, *235*, 769.

(26) Crompton, T. R. "Chemical Analysis of Organometallic Compounds"; Academic Press: New York, 1975; Vol. 4, p 12.

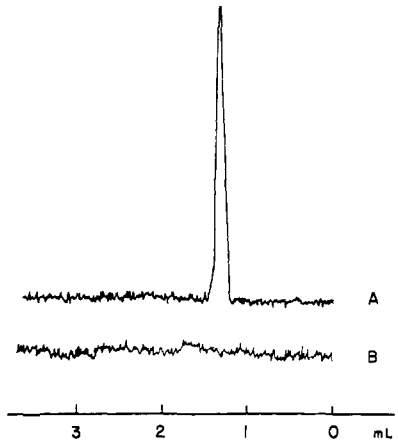

Figure 6. Aqueous gel permeation chromatograms of polymerized (A) and nonpolymerized (B) vesicles of **5**; identical quantities of lipid were injected.

on standing after 48 h, polymerized preparations were stable for more than 2 weeks. Second, ethanol, which is known to destroy liposomes, substantially reduces the apparent turbidity and, presumably, the vesicle population of nonpolymerized **5** (Figure 5).[27] In contrast, the turbidity for polymerized analogues showed no significant dependence on added ethanol over a similar concentration range. These results are fully consistent with the permeability data for **5** in 20% ethanol (v/v) (Table I). Third, attempted aqueous gel permeation chromatography of nonpolymerized vesicles of **5** failed to show any high molecular weight component (Figure 6). This is presumably due to disruption of the vesicles under the high-pressure liquid chromatographic conditions employed. When an identical concentration of lipid and an identical injection volume were used, polymerized vesicles of **5** produced an intense peak in the molecular weight region close to blue dextran (ca. 2×10^6). Taken together, these results provide very strong evidence that polymerization increases vesicle stability.

The phosphatidylcholine vesicles described herein represent members of a unique polymer class which should find broad use. Further studies, now underway in our laboratories, are aimed at synthesizing closely related analogues and examining the potential value of these materials as drug carriers.

Experimental Section

General Methods. Unless stated otherwise, all reagents and chemicals were obtained commerically and used without further purification. Tetrahydrofuran (Aldrich Chemical Co., Gold Label) was purified by distillation from sodium and benzophenone under a nitrogen atmosphere. Pyridine (Aldrich Chemical Co., Gold Label) was used as obtained. Deionized water or deuterium oxide (Aldrich Chemical Co., Gold Label) was distilled twice under a nitrogen atmosphere and stored under nitrogen. The following chemicals or materials used in this work are listed along with their commercial source: palmitoyl-L-α-lysophosphatidylcholine (Sigma Chemical Co.); 12-hydroxydodecanoic acid, methacryloyl chloride, 4-(dimethylamino)pyridine, ethyl chloroformate, dioxane (Gold Label), chloroform, azobis(isobutyronitrile) (Aldrich Chemical Co.); tetrabutylammonium hydroxide, 25% in CH_3OH (J. T. Baker); [^{14}C]-sucrose, 360 mCi/mmol, 20% ethanol solution (ICN Laboratories); Omnifluor (New England Nuclear); Spectrapor dry membrane tubing, cylindrical diameter of 14.6 mm, 6000–8000 (Spectrum Medical Industries); Sephadex G-50-150 (Sigma Chemical Co.); silica gel for chromatography, Type 60, 70–230 mesh (Merck); silica analytical TLC plates, Type 60, F-254 (Merck); AG-501-X8(D) resin (Bio-Rad Laboratories); *Crotalus adamanteus* (Miami Serpentarium Laboratories); palmitoyl anhydride was prepared from the corresponding carboxylic acid (Eastman Organic Chemicals).

All Fourier transform ^{1}H NMR spectra were recorded in D_2O at 25 °C (500 scans) with a JEOL FX 60Q instrument. Other ^{1}H NMR, IR, and UV spectra were recorded with Varian A-60, Beckman Acculab 7, and Bausch and Lomb Spectronic 20 (or McPherson GCA) spectrometers, respectively. High-pressure liquid chromatograms were obtained with a Waters ALC/GPC-201 instrument, equipped with a Model 6000A solvent delivery system, differential refractive index detector, U6K injector, and a μ-Bondagel (high Å) column. Distilled water was the eluent. Sonication was performed by using a Heat Systems Model W-225R sonicator (bath type). Photopolymerization was carried out with a Rayonet photochemical reactor (Southern N.E. Ultraviolet Co.) equipped with 16 2537-Å Rayonet photochemical reactor lamps. Electron micrographs were recorded on a Philips 400 TEM microscope. All vesicle samples were prepared under nitrogen in a Burlitch inert-atmosphere apparatus (Ace Glass Co.).

Samples for electron microscopy were prepared on 200-mesh carbon-coated copper grids (Ted Pella Inc.) by the "Drop" method.[12] The liquid scintillation cocktail used was derived from 8.0 g of Omnifluor (98% 2,5-diphenyloxazole + 2% *p*-bis[*o*-methylstyryl)benzene] dissolved in 1 L of spectrograde dioxane (Aldrich Chemical Co.). Liquid scintillation was performed with a Nuclear Chicago Mark II instrument, Model 4643.

12-(Methacryloyloxy)dodecanoic Acid (1). 12-Hydroxydodecanoic acid (2.16 g, 10 mmol) was dissolved in 30 mL of THF and placed in a 100-mL round-bottomed single-necked flask equipped with a Teflon-coated magnetic stirring bar. After addition of pyridine (1.09 g, 15 mmol), the solution was cooled to 0 °C, and methacryloyl chloride (1.04 g, 10 mmol) in 15 mL of THF was added dropwise through a pressure-equalized addition funnel. The contents were then warmed to room temperature and stirred for an additional 12 h. Removal of solvent under reduced pressure (room temperature) afforded a semisolid. The crude ester was dissolved in 25 mL of ether, the mixture was washed with distilled water, and the ether layer was then dried (anhydrous $MgSO_4$). Evaporation of ether followed by chromatographic purification on a silica gel column (2.5 × 40 cm) (1:2 ethyl acetate–hexane) furnished 1.50 g (53%) of **1**: NMR ($CDCl_3$) δ 1.3 (br s, 18 H, $(CH_2)_9$), 1.95 (m, 3 H, $C{=}CCH_3$), 2.15–2.50 (m, 2 H, CH_2CO_2H), 3.9–4.3 (m, 2 H, OCH_2), 5.52 (m, 1 H, vinyl), 6.08 (m, 1 H, vinyl), 11.2 (br s, 1 H, CO_2H); IR (neat) $\nu_{C=O}$ 1720, 1735, $\nu_{C=C}$ 1640 cm^{-1}.

12-(Methacryloyloxy)dodecanoic Anhydride (2) To a solution of **1** (0.753 g, 2.65 mmol) dissolved in 15 mL of THF was added 0.5 mL (3 mmol) of triethylamine. The resulting solution was cooled to −20 °C, and a solution of ethyl chloroformate (0.3 mL, 3.1 mmol) in 10 mL of THF was added to it in a dropwise fashion. The mixture was then stirred for 2 h, warmed to room temperature, and stirred for an additional 20-min period. The contents were then cooled to −20 °C and a solution of 0.753 g of **1** plus 0.5 mL of triethylamine dissolved in 15 mL of THF was added dropwise. After the product mixture was stirred overnight at room temperature, the solvent was removed under reduced pressure (room temperature). The crude anhydride was dissolved in ether (250 mL), and the ether layer was washed with water and dried (anhydrous $MgSO_4$). Removal of ether followed by extensive drying [25 °C, 24 h (0.1 mm)] afforded 1.37 g (94%) of **2**: NMR ($CDCl_3$) δ 1.3 (br s, 36 H, methylene), 1.96 (m, 6 H, $C{=}CCH_3$), 2.2–2.6 (m, 4 H, CH_2CO), 3.9–4.5 (m, 4 H, OCH_2), 5.52 (m, 2 H, vinyl), 6.08 (m, 2 H, vinyl); IR (neat $\nu_{C=O}$ 1730, 1800 (anhydride), $\nu_{C=O}$ 1720 (ester), $\nu_{C=C}$ 1630 cm^{-1}.

L-α-Glycerophosphorylcholine. The combined egg yolks from 12 eggs were separated and washed thoroughly with acetone until the washings were colorless. The resulting off-white crystalline solid was extracted with 2 × 100 mL of 2:1 $CHCl_3$–CH_3OH. Each extraction step was carried out with vigorous stirring for 2 h. The extracts were combined and concentrated under reduced pressure (room temperature), yielding ca. 22 g of waxy product. Chromatographic purification was then carried out on 200 g of neutral alumina, initially with $CHCl_3$ and 9:1 $CHCl_3$–CH_3OH as eluting solvents. After all yellow products were removed from the column, egg lecithin was eluted with 1:1 $CHCl_3$–CH_3OH. The final product (7.8 g) had an R_f of 0.35 (silica gel, 65:35:4 $CHCl_3$–CH_3OH –H_2O), which was identical with an authentic sample.

Hydrolysis of the lecithin (7.8 g) was carried out by dissolving it in 70 mL of ether, removing the suspended particles by centrifugation, adding 7.8 mL of 0.1 M tetrabutylammonium hydroxide (methanol solution), and shaking the mixture occasionally by hand. After L-α-glycerophosphorylcholine precipitated out of solution, the liquid phase was decanted. The lipid was then washed with ether and dried [25 °C (0.1 mm), 24 h]. The product thus obtained was either used directly in further synthesis or stored in CH_3OH at −15 °C.

Bis[12-(methacryloyloxy)dodecanoyl]-L-α-phosphatidylcholine (3). A methanolic solution of L-α-glycerophosphorylcholine (0.488 g, 1.89 mmol) was placed in a 50-mL single-necked, round-bottomed flask, the methanol was removed under reduced pressure, and the residue was dried [25 °C (0.1 mm) 24 h]. To this dried sample was added 3.12 g (5.67 mmol) of **2** dissolved in 20 mL of $CHCl_3$ (freshly distilled over P_2O_5). While the resulting solution was vigorously stirred with a Teflon-coated magnetic stirring bar, 4-(dimethylamino)pyridine (0.691 g, 5.67 mmol) was added. The contents of the flask was then degassed with nitrogen, stoppered, protected from light, and stirred for 72 h at room temperature.

(27) Tran, C. D.; Klahn, P. L.; Romero, A.; Fendler, J. H. *J. Am. Chem. Soc.* **1978**, *100*, 1622.

Chloroform was then removed under reduced pressure (room temperature), and the residue was dissolved in 5 mL of 4:5:1 $CHCl_3$–CH_3OH–H_2O and passed through an AG-501-X8(D) resin (1 × 17 cm). The column was washed with 20 mL of the same solvent, and the eluted product was combined followed by solvent evaporation. The crude product was then dissolved in a minimum volume of $CHCl_3$ and further purified by chromatography using a column of silica gel (1 × 20 cm). Elution was performed by using the following solvent systems in succession: $CHCl_3$, 9:1 $CHCl_3$–CH_3OH, 1:1 $CHCl_3$–CH_3OH, and 1:9 $CHCl_3$–CH_3OH. Fractions were analyzed by TLC (silica, 65:25:4 $CHCl_3$–CH_3OH–H_2O), and those fractions containing a product with an R_f similar to egg lecithin were combined and the solvent was evaporated. Lipid **3** thus obtained (0.534 g, 36%) showed only one spot by TLC, R_f 0.36 (silica gel, 65:25:4 $CHCl_3$–CH_3OH–H_2O): IR ($CHCl_3$) ν_{OH} 3200–3600, $\nu_{C=O}$ 1720 (b), $\nu_{C=C}$ 1640, $\nu_{+N(CH_3)_3}$ 1060, 1090, 975 cm^{-1}; NMR ($CDCl_3$) δ 1.3 (br s, 36 H, (CH_2)), 1.93 (m, 6 H, $C{=}CCH_3$), 2.2–2.45 (m, 4 H, CH_2COO), 3.38 (br s, 9 H, $^+N(CH_3)_3$), 3.75–4.5 (m, 13 H, CH_2O and CH_2N^+), 5.52 (m, 2 H, vinyl), 6.08 (m, 2 H, vinyl). Anal. Calcd for $C_{40}H_{72}NO_{12}P$: N, 1.77; P, 3.93. Found: N, 1.79; P, 3.7.

Similar yields of **3** were obtained when a $CdCl_2$ complex of L-α-glycerophosphorylcholine was used.

1-[12-(Methacryloyloxy)dodecanoyl]-2-palmitoyl-L-α-phosphatidylcholine (4). Phosphatidylcholine **3** was hydrolyzed by procedures similar to those described by Chakrabarti and Khorana[20] to yield 12-(methacryloyloxy)dodecanoyl-L-α-lysophosphatidylcholine. Lipid **3** (0.459 g, 0.58 mmol) was dissolved in a mixture of 0.5 mL of absolute alcohol and 4.5 mL of diethyl ether. To this solution was added 1 mg of crude snake venom (*Crotalus adamanteus*) dissolved in 0.05 mL of 0.005 M $CaCl_2$ (aqueous solution). The course of the hydrolysis was monitored by TLC (silica, gel, 65:25:4 $CHCl_3$–CH_3OH–H_2O). After 4 h, no lecithin could be detected and the reaction mixture was quenched by addition of 5 mL of water. The ether layer, which contained the organic acid, was removed and the aqueous layer washed extensively with ether. After the aqueous layer was lyophilized, the residue was dissolved in 2:1 $CHCl_3$–CH_3OH and centrifuged. The resulting clear solution, which was free of lecithin (TLC), was evaporated, yielding 0.300 g of 12-(methacryloyloxy)dodecanoyl-L-α-lysophosphatidylcholine.

Into a 25-mL round-bottomed flask equipped with a Teflon-coated stirring bar was placed 0.300 g (0.5 mmol) of 12-(methacryloyloxy)dodecanoyl-L-α-lysophosphatidylcholine plus 0.494 g (1.0 mmol) of palmitoyl anhydride dissolved in 15 mL of $CHCl_3$ (freshly distilled over P_2O_5). 4-(Dimethylamino)pyridine (0.122 g, 1 mmol) was then added, and the mixture was degassed with nitrogen and stirred at room temperature in the dark for 36 h. The course of the reaction was monitored by TLC (silica gel, 65:25:4 $CHCl_3$–CH_3OH–H_2O). The stirring bar was then removed and the solvent evaporated under reduced pressure. The residue was dissolved in a minimum volume of 4:5:1 $CHCl_3$–CH_3OH–H_2O and the solution passed through an AG-501-X8(D) column (1 × 20 cm). The remaining adsorbed lecithin was eluted with 20 mL of the same solvent. The eluted product was then combined followed by solvent evaporation under reduced pressure (room temperature). The lecithin was further purified by chromatography using a silica gel column (1 × 15 cm) and successive elution with $CHCl_3$ and $CHCl_3$–CH_3OH (gradient of 9:1 to 1:9). Fractions containing the lecithin (R_f similar to that of egg lecithin) were collected, and the solvent was removed under reduced pressure, affording 0.264 g (68%) of **4**: R_f 0.36 (silica gel, 65:25:4 $CHCl_3$–CH_3OH–H_2O); IR ($CHCl_3$) ν_{OH} 3200–3500, $\nu_{C=O}$ 1720, 1740, $\nu_{C=C}$ 1640, $\nu_{+N(CH_3)_3}$ 1100, 1170, 975 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_3CH_2), 1.25 (s, 44 H, CH_2), 1.93 (m, 3 H, $C{=}CCH_3$), 2.2–2.5 (m, 4 H, CH_2COO), 3.38 (br s, 9 H, $(CH_3)_3N^+$), 3.68–4.3 (m, 11 H, CH_2O and $-CH_2N^+$), 5.5 (m, 1 H, vinyl), 6.08 (m, 1 H, vinyl). Anal. Calcd for $C_{40}H_{76}NO_{10}P$: N, 1.84; P, 4.06. Found: N, 1.71; P, 3.91.

Dipalmitoyl-L-α-phosphatidylcholine. By procedures similar to those described for the synthesis of **3**, L-α-glycerophosphorylcholine (0.454 g, 1.76 mmol) was reacted with 3.06 g (6.18 mmol) of palmitoyl anhydride in the presence of 0.754 g (6.18 mmol of 4-(dimethylamino)pyridine. The isolated yield of dipalmitoyl L-α-phosphatidylcholine was 0.500 g (39%). The TLC behavior and IR spectrum were identical with those of an authentic sample.

1-Palmitoyl-2-[12-(methacryloyloxy)dodecanoyl]-L-α-phosphatidylcholine (5). By procedures similar to those described for the synthesis of **4**, 0.367 g (0.5 mmol) of dipalmitoyl-L-α-phosphatidylcholine was converted into 0.217 g (88%) of 1-palmitoyl-L-α-lysophosphatidylcholine. Reaction of 50 mg (0.101 mmol) of this lysolecithin with 83.6 mg (0.152 mmol) of **2** in 10 mL of $CHCl_3$ in the presence of 18 mg (0.149 mmol) of 4-(dimethylamino)pyridine for 72 h afforded 0.063 g (83%) of **5**. TLC behavior and NMR and IR spectra were identical with those of **4**. Anal. Calcd for $C_{40}H_{76}NO_{10}P$: N, 1.84; P, 4.06. Found: N, 1.71; P, 4.24.

Vesicle Preparation. Typically, 15 mg of lipid was placed in a 13 × 100 mm Bausch and Lomb UV cell (for use with Spectronic 20 instruments), which, in turn, was placed in a two-necked, 250-mL round-bottomed flask. The flask was then connected to an inert-atmosphere apparatus (Burlitch type) Ace Glass Co. and degassed with nitrogen. Deuterium oxide (3 mL) that had been degassed under nitrogen was added to this sample, and the cell was then sealed with a No-Air stopper. The cell was placed in a *bath-type* sonicator at 50 °C, and the sample was irradiated at a power level of 125 W. After 5 min of sonication, the tube was removed from the bath, manually shaken for 5 min, and returned to the sonicator for additional irradiation (normally 1 h). The sonication was monitored by measuring the turbidity at 400 nm; transmittance was usually at least 70% for the final preparation. Very small quantities of nondispersed lipid were removed by centrifugation with an International clinical centrifuge.

The Fourier transform ^1H NMR spectra of vesicle dispersions prepared from **3**, **4**, or **5** clearly showed the presence of the intact vinylidene group. Extraction of a vesicle sample of **5** with 2 mL of chloroform followed by solvent removal under reduced pressure afforded nearly quantitative recovery of **5**, whose IR spectrum (Nujol or $CHCl_3$) was identical with that of the starting lipid.

Vesicle Polymerization. Vesicle preparations were transferred to 10-mL Vycor tubes (under a nitrogen atmosphere) and photopolymerized by UV irradiation (254 nm, Rayonet photochemical reactor). The course of the polymerization was conveniently monitored by following the disappearance of the allyl protons in the Fourier transform ^1H NMR spectrum. In the case of **5**, vesicle polymerization was complete after 1 h of irradiation. Attempted extraction with 2 mL of $CHCl_3$ failed to produce any organic-soluble lipid monomer or polymer.

Entrapment and Leakage of [^{14}C]Sucrose. [^{14}C]Sucrose was entrapped in vesicles derived from lipids **3**–**5** by procedures similar to those described for the vesicle preparation. In this case, however, 5 mg of lipid was suspended in 1.0 mL of distilled water containing 1 μCi of [^{14}C]sucrose. Nonentrapped sucrose was removed by gel filtration on Sephadex G-50-150; 1.0-mL fractions were eluted every minute. Typically, 1 mL of the suspension was added to a wet 30 × 1 cm column and then eluted with distilled water. Vesicles recovered in the void volume of the column were immediately placed in presoaked seamless cellulose bags and were dialyzed against 50 mL of water (or an appropriate solution) generally at 23 °C. Mild stirring was used in all dialysis experiments. Samples (100 μL) were withdrawn periodically from the bag, added to 3.0 mL of the scintillation cocktail, and analyzed directly for [^{14}C]sucrose by liquid scintillation counting. After each sampling, the bag was placed in a fresh beaker containing 50 mL of water (or aqueous solution). For those experiments which were carried out with 0.154 M NaCl, 5 mL of the cocktail was employed.

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