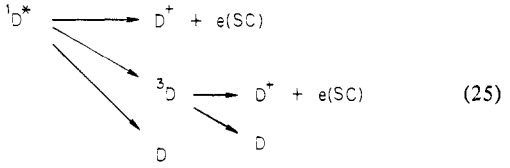
The energy and the lifetime of excited dimers are main determining factors of φ_{el}^{D}. It has been said that an intersystem crossing from the excited singlet state to the triplet state efficiently occurs in the dimers of dyes.[26] Then, it is necessary to taken into account the triplet state of the dimers to deal with the electron injection from the dimers. The process in eq 17 is modified as follows:



(25)

Electron injection following the yield of ^3D would occur when the rate of intersystem crossing $^1D^* \rightarrow {}^3D$ is somewhat larger than the rate of electron injection from $^1D^*$. The presence of this process can be checked through the measurement of transient behavior of the photocurrent and/or through that of the phosphorescence of ^3D on the surfaces. Such experiments are of interest to reveal the basic photochemical processes of dyes at solid surfaces.

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Registry No. RhB, 81-88-9; RhB dimer, 14728-79-1; SnO_2, 18282-10-5; TiO_2, 13463-67-7.

Permeability Characteristics of Polymeric Bilayer Membranes from Methacryloyl and Butadiene Lipids

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Abstract: Four polymerizable lipids were synthesized and used for the formation of synthetic bilayer membranes (vesicles). Two of the lipids were methacryloyl ammonium lipids, one with a methacrylamide at the hydrophilic head group of the lipid (α-MA) and one with a methacrylate group at the hydrophobic tail of the lipid (ω-MA). Thermally initiated polymerization of the monomeric bilayer vesicles gave polymers with retention of vesicle structure. The size distribution of the aqueous suspension was not altered significantly on polymerization, and the membranes continued to entrap [^{3}H]glucose. The permeability of poly(α-MA) and poly(ω-MA) membranes is about half that of the unpolymerized bilayers. Previously we reported that about 500 monomer units were found per average polymer chain of poly(α-MA) and poly(ω-MA), which shows that there are several (20 to 100) polymer chains per vesicle (Dorn, K., et al. *Makromol. Chem., Rapid Commun.* **1983**, *4*, 513). Two butadiene lipids, one based on a phosphatidylcholine (PC) structure, and one with a taurine head group, also formed bilayer membranes, which could be photopolymerized by exposure to ultraviolet light. These lipids have a sorbate unit (λ_{max} 257 nm) in each of the two hydrocarbon chains, which allows the photopolymerization to proceed with the formation of cross-links. Poly(butadiene PC) membranes effectively entrapped [^{3}H]glucose for at least a week and were not disrupted by the use of the surfactant Triton X-100.

Introduction

Synthetic membrane liposomes or vesicles have been a topic of increasingly active research since the early 1960s. These spherical membrane models are composed of a bilayer(s) of lipid that encloses an aqueous volume.[1] In addition to their use as membrane models, vesicles are of interest for their ability to sequester or encapsulate reagents,[2,3] for the separation of charges and charged species in solar energy conversion,[4,5] and for the effect of the lipid bilayer organization on chemical reactions.[6,7] Frequently, vesicles are prepared from phospholipids, either naturally occurring or synthetically accessible lipids. More recently Kunitake and co-workers introduced the concept of a totally synthetic bilayer membrane of dimethyldidodecylammonium bromide.[8] Since 1977, several new bilayer-forming materials have been synthesized, including double-chain cationic, anionic, and zwitterionic amphiphiles and single-chain amphiphiles based on liquid-crystal materials.[9-11]

In 1980 and 1981, several groups reported the synthesis of polymerizable lipids and their incorporation into and polymerization in vesicles. Regen et al.[12] reported a cationic amphiphile with a methacrylate at the terminus of one hydrophobic chain. In short order, several lipid diacetylenes[7,13-15] were described. More recently, butadiene lipids,[6,16] additional methacryloyl lip-

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ids,[17,18] and vinyl lipids[19] have been introduced. Polymerizable α,ω-dipolar amphiphiles with a diacetylene moiety have also been studied.[20]

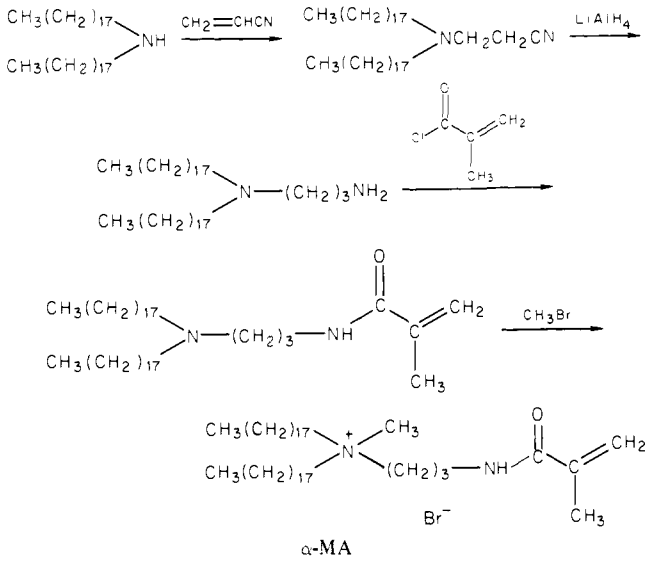
The conversion of a spherical bilayer membrane (vesicle) from a self-organized array of several thousand lipid molecules into a structure containing several polymer chains has been casually termed the formation of polymeric vesicles.[12] To date, these materials have generally been characterized by spectroscopic methods to indicate the extent of polymer formation, by electron microscopic images, and by enhanced stability to surfactants and organic solvents. It is expected that polymer formation in bilayer membranes will alter the permeability characteristics of the membrane and hence change their ability to sequester molecules.

We present here the first kinetic analysis of the permeability properties of bilayer membranes before and after polymer formation. These data are coupled with information on the size of the dispersions and the molecular weight of the polymers formed in these bilayer membranes, to further characterize the vesicle structures.

Experimental Section

D-[^{3}H]Glucose (New England Nuclear) had an activity of ~30 Ci/mmol. Dimethyldioctadecylammonium bromide (DODAB) (Kodak Laboratory Chemicals) was recrystallized twice from acetone.

α-Methacryloyl Ammonium Lipid (α-MA). Dioctadecylamine (20 g, 38 mmol) (Fluka) in 200 mL of acrylonitrile (freshly distilled) was refluxed for 18 h. The excess solvent was evaporated under vacuum, and



α-MA

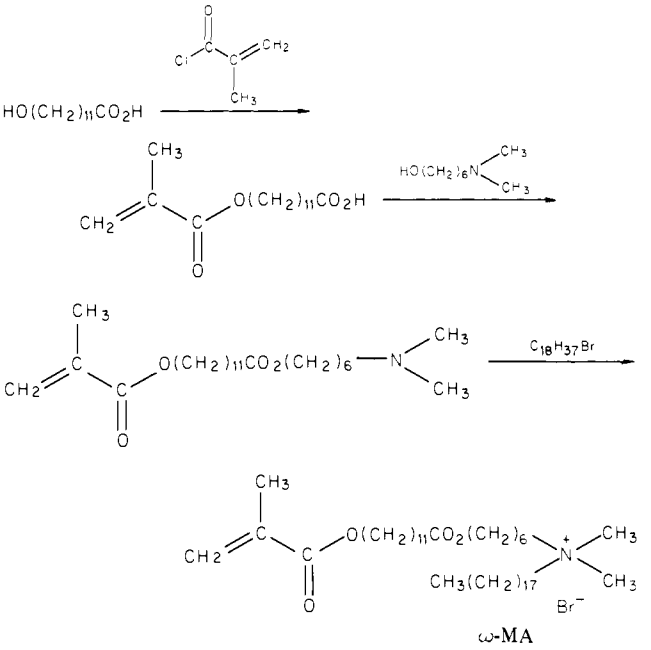
the waxy, slightly yellow semisolid was dissolved in 2:1 petroleum ether (PE)/ether (Et_2O) and passed over a 200-g silica gel column. The solvent was evaporated, and the residue was recrystallized twice from 100 mL of acetone: yield 16 g (75%); mp 40 °C.

Lithium aluminum hydride (5.3 g, 140 mmol) was dispersed in 150 mL of dry Et_2O, and 19.5 g (34 mmol) of the nitrile dissolved in 50 mL of dry Et_2O was added dropwise so that the solution refluxed without external heating. After addition was complete, the reaction was refluxed for 4 h, or until no starting compound was seen by TLC (1:3 ethyl acetate/*n*-hexane). Water was added dropwise until hydrogen formation ceased (ice cooling). The Et_2O phase was separated, 5 g of $NaHCO_3$ was added, and the water phase was extracted three times with 50 mL of Et_2O. The precipitated $Al(OH)_3$ was extracted twice with 150 mL of hot diisopropyl ether, and the combined ether phases were dried with Na_2SO_4 and evaporated: yield 17 g (87%); mp 45–47 °C. The crude amine was used without further purification.

The amine (4.6 g, 8 mmol), 1.1 mL of triethylamine, and 5 mg of 2,6-di-*tert*-butyl-*p*-cresol (inhibitor) were dissolved in 100 mL of dry dichloromethane. At 0 °C, 0.83 g (8 mmol) of methacryloyl chloride (1:1 solution in dichloromethane) was added, and the mixture was stirred for 15 min at 0 °C and 2 h at room temperature. The solution was extracted three times with water, dried, and evaporated. The crude product (5.5 g) was dissolved in 12 mL of chloroform, placed on a 150-g silica gel column, and eluted with ethyl acetate (R_f 0.3). The product was recrystallized from acetone: yield 2 g (39%); mp 35–37 °C.

The methacrylamide (3.4 g, 5.3 mmol) and 5 mg of 2,6-di-*tert*-butyl-*p*-cresol were dissolved in 70 mL of dry dichloromethane. At −10 °C, 12 mL (125 mmol) of methyl bromide was added. The reaction mixture was stirred for 4 h with ice cooling and kept overnight at room temperature. The solvent was evaporated, and the product was dissolved in 95:5 chloroform/methanol and eluted from a 100-g silica gel column with the same solvent. Recrystallization from acetone gave 1.2 g (35%): mp 104–110 °C; R_f (4:1 chloroform/methanol) 0.8; NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3–1.7 (m, 64 H, CH_2), 2.0 (s, 3 H, CH_3), 2.2 (t, 2 H, CH_2), 3.3 (s, 3 H, CH_3N^+), 3.3–3.5 (m, 6 H, CH_2N^+), 3.7 (m, 2 H, CH_2NH), 5.4 (s, 1 H, CH=C), 6.1 (s, 1 H, CH=C), 8.0 (t, 1 H, NHCO); IR (KBr) $\nu_{C=C}$ 1645 cm^{-1}, ν_{NHCO} 1600, 1540 cm^{-1}.

ω-Methacryloyl Ammonium Lipid (ω-MA). 12-Hydroxydodecanoic acid (4.3 g, 20 mmol), 5 mg of 2,6-di-*tert*-butyl-*p*-cresol, and 2.2 g (30 mmol) of pyridine were dissolved in 100 mL of dry tetrahydrofuran. At 0 °C, 1.85 mL (20 mmol) of methacryloyl chloride was added dropwise, and the reaction mixture was stirred overnight at room temperature.



ω-MA

The precipitate was filtered off, and the filtrate was evaporated under vacuum at room temperature. The residue was dissolved in Et_2O, washed with water, dried, and evaporated. The crude product was applied to a 400-g silica gel column and eluted with 1:2 ethyl acetate/*n*-hexane. In the presence of inhibitor, the collected fractions were evaporated under vacuum at room temperature, then dried for 2 h in a vacuum desiccator: yield 2.3 g (40.5%). The TLC in 1:2 ethyl acetate/*n*-hexane showed one spot (R_f 0.3).

The methacrylate (2.3 g, 8 mmol), 1.2 g (8 mmol) of 6-dimethylaminohexan-1-ol, 30 mg of dimethylaminopyridine, and 5 mg of 2,6-di-*tert*-butyl-*p*-cresol were dissolved in 20 mL of dry dichloromethane. At 0 °C, 1.8 g (9 mmol) of dicyclohexylcarbodiimide (DCC) was added, and the reaction mixture was stirred at 0 °C for 30 min and 2 h at room temperature. The precipitate was filtered off, and the solvent was evaporated (room temperature). The residue was applied to a 400-g silica gel column and eluted with 100:15:1 chloroform/methanol/aqueous ammonia. The fractions (R_f 0.6) were evaporated in the presence of inhibitor at room temperature and dried for 2 h in a vacuum desiccator: yield 1.2 g (36%) of a slightly yellow oil. The product was used without further characterization.

The long-chain amine (800 mg, 2 mmol) was quaternized with 1.3 g (4 mmol) of octadecyl bromide, 10 mg of 2,6-di-*tert*-butyl-*p*-cresol in 10 mL of acetone was added, and the mixture was stirred at 50 °C for 2–3 days. The product was purified on a silica gel column. The fractions (R_f 0.3–0.4) were evaporated at room temperature and dried for 2 h in a

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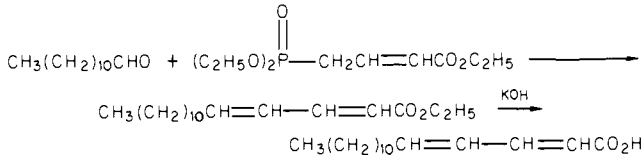
vacuum desiccator: yield 370 mg (25%); mp 37 °C; NMR ($CDCl_3$) δ 0.9 (t, 3 H, CH_3), 1.3–1.7 (m, 58 H, CH_2), 1.9 (t, 3 H, CH_3), 2.3 (t, 2 H, CH_2CO_2), 3.4 (s, 6 H, CH_3N^+), 3.4–3.6 (m, 4 H, CH_2N^+), 4.1 (t, 4 H, OCH_2), 5.5 (t, 1 H, CH=C), 6.1 (s, H, CH=C); IR (KBr) $\nu_{C=O}$ 1730, 1710 cm^{-1}, $\nu_{C=C}$ 1635 cm^{-1}.

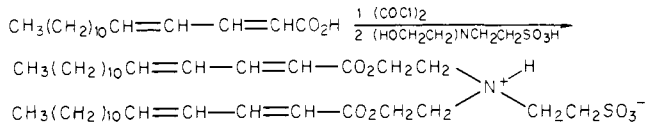
Ethyl Hexadeca-*trans*-2,*trans*-4-dienoate. A suspension of 2.4 g (50 mmol) of 50% sodium hydride mineral oil dispersion in 100 mL of dry THF was stirred at 0 °C as 12.5 g (0.05 mL) of triethyl 4-phosphonocrotonate was added dropwise over 45 min. The mixture was stirred in the cold for 45 min, and 9.2 g (50 mmol) of dodecanecarboxaldehyde was added dropwise with stirring over 1 h. The reaction mixture was stirred at 0 °C for 0.5 h and at room temperature for 0.5 h before it was diluted to 1 L with water. The product was extracted by stirring with Et_2O and ligroin. The combined extracts were dried (Na_2SO_4) and evaporated: yield 10.5 g.

2,4-Hexadecadienoic Acid. Ethyl 2,4-hexadecadienoate (7.5 g) was added to a solution of 3.75 g of potassium hydroxide in 50 mL of methanol, and the mixture was heated at gentle reflux for 2 h. The



mixture was cooled to room temperature, placed in a separatory funnel, diluted with water, and washed by swirling with ligroin. The aqueous layer was acidified with hydrochloric acid, stirred for 0.5 h, and chilled in a freezer. The solid was collected and recrystallized from a small amount of ligroin.

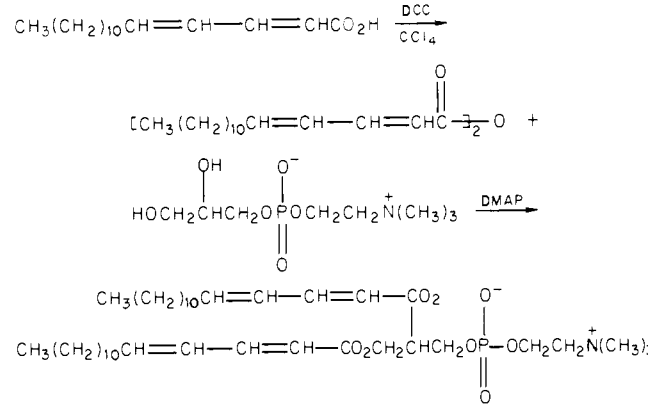
***N*,*N*-Bis[2,4-hexadecadienoylcarbonylethyl]-2-sulfoethylamine (Butadiene Taurine).** 2,4-Hexadecadienoic acid (1.34 g, 5.3 mmol) was added to 15 mL of oxalyl chloride, and the solution was heated at gentle reflux for 2 h. The excess oxalyl chloride was removed under reduced pressure,



first with a rotary evaporator, then with a vacuum pump. The acid chloride was taken up in 10 mL of dry dichloromethane and added dropwise to a stirred suspension of 0.48 g (2.25 mmol) of *N*,*N*-bis(2-hydroxyethyl)-2-sulfoethylamine and 0.70 g (5.7 mmol) of 4-dimethylaminopyridine in 15 mL of dry dichloromethane. The flask was stoppered, and the mixture was stirred at room temperature for 65 h. The reaction mixture was heated at gentle reflux for 0.75 h and cooled, and the solvent was evaporated. The residue was chromatographed on silica gel with a dichloromethane/methanol gradient: NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3 (br, s, 36 H, CH_2), 2.1 (s, 4 H, CH_2C=C), 2.7–3.6 (m, 8 H, $(CH_2)_2NCH_2CH_2SO_3H$), 4.2 (s, 4 H, CH_2CO), 5.7 (d, 2 H, J = 15 Hz, C=$CHCO_2$), 5.8–6.3 (m, 4 H, C*H*=C*H*CH_2), 7.0–7.4 (m, 2 H, CH=CCO_2).

2,4-Hexadecadienoic Anhydride. 2,4-Hexadecadienoic acid (4.78 g, 19 mmol) was dissolved in 45 mL of dry carbon tetrachloride. Dicyclohexylcarbodiimide (2.15 g, 10 mmol) was added, and the reaction mixture was allowed to stand at room temperature for 24 h with occasional swirling. The precipitate was removed by filtration, and the filtrate was evaporated to dryness. The residue was taken up in a small amount of ether and filtered through a Whatman No. 5 paper. The filtrate was chilled, and the precipitated solid was collected and dried. The solid was chromatographed on silica gel eluted with dichloromethane: yield 2.64 g.

1,2-Bis(2,4-hexadecadienoyl)-*sn*-glycero-3-phosphorylcholine (Butadiene Phosphatidylcholine) by 1,2-Diacylation of L-α-Glycerophosphorylcholine.[21] The L-α-glycerophosphorylcholine–cadmium chloride complex (0.48 g, 1 mmol) was dried by repeated (4×) evaporation of dry benzene. The residue was suspended in 25 mL of dry, stabilizer-free, freshly distilled chloroform. The anhydride (1.22 g, 2.5 mmol) and 4-dimethylaminopyridine (0.24 g, 2.0 mmol) were added, and the reaction flask was flushed with dry nitrogen and tightly sealed. The reaction mixture was stirred magnetically at room temperature for 40 h. The solvent was evaporated, and ~30 mL of a 5:4:1 mixture of methanol, chloroform, and water was added. The insoluble material was removed by filtration, and the filtrate was poured through a column of Rexyn I-300 resin (50 mL). The resin was washed with ~150 mL of the same



solvent. The combined effluents were evaporated, and the residue was chromatographed on 100–200 mesh activated silica gel. Several impurities were removed with a chloroform/methanol gradient, and the product was eluted with pure methanol: yield 0.54 g: NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3 (br s, 36 H, CH_2), 2.2 (s, 4 H, CH_2C=C), 3.4 (s, 9 H, CH_3N^+), 3.6–4.8 (m, 8 H, $CH_2CH_2OPO_3CH_2$, CH_2CO_2), 5.3 (br s, 1 H, $CHCO_2$), 5.7 (d, 2 H, J = 15 Hz, C=$CHCO_2$), 5.9–6.4 (m, 4 H, C*H*=C*H*CH_2), 7.0–7.4 (m, 2 H, CH=C-CO_2).

Sample Preparation. Lipids (12–14 mg) were weighed into a 10-mL polycarbonate tube and hydrated with 1.2–1.4 mL of doubly distilled water (the butadiene lipids were dispersed in 10 mM of Hepes buffer, pH 8.0). The dispersion was sonicated with a Heat Systems cuphorn sonifier at 50 °C: α-MA, 15 min, 50 W; ω-MA, 5 min, 50 W; butadiene taurine, 20 min, 50 W; butadiene PC, 15 min, 40 W; DODAB, 15 min, 50 W.

Samples for glucose permeability were prepared as follows. A 1-μL portion of the [^{3}H]glucose stock solution (9:1 ethanol/water) was evaporated to dryness with argon in a graduated test tube. The sonicated vesicle solution (1.2 mL) was added, and the mixture was sonicated in a bath-type sonicator for 30 min at room temperature to speed diffusion of [^{3}H]glucose into the vesicles. This solution of labeled vesicles was divided in half. The monomer membranes were kept at room temperature overnight before gel permeation chromatography. The butadienes were handled under red light. The second half handled the sample was polymerized.

Polymerization of Methacryloyl Lipids. Azobisisobutyronitrile (AIBN) Initiator. The appropriate amount of an AIBN stock solution in $CHCl_3$ to give an initiator/ monomer ratio of 0.1 was evaporated with argon in a screw-cap glass vial. The vesicle preparation was added to the dried initiator, and the mixture was sonicated in a bath sonicator for 1–2 min to disperse the initiator. The sample was flushed with argon for 30 min. The glass vial was closed under argon and transferred to a constant-temperature bath. Polymerization was carried out at 60–65 °C for 15–18 h. The sample was equilibrated at room temperature for 1–2 h before gel permeation chromatography.

2,2′-Azobis(2-amidinopropane hydrochloride) (AAPH) Initiator. The appropriate amount of an AAPH stock solution in water was added to the vesicle samples, and the samples were sonicated for 1–2 min in a bath sonicator to ensure equal distribution of the initiator in the sample. The sample was flushed with argon and polymerized as described above.

Polymerization of Butadiene Lipids. The vesicle suspension was transferred to a 1-cm quartz cuvette with a tight Teflon stopper. The sample was flushed with argon and the cuvette was placed horizontally under a bank of UV lamps and irradiated for 3 h at room temperature. A 0.6-mL sample gave a 1-mm thick fluid in the horizontal cell.

Gel Permeation Chromatography. The monomer or polymer vesicles were chromatographed on a Sephadex G50 column (1.6 × 40 cm) at room temperature to separate the vesicles and entrapped [^{3}H]glucose from the free [^{3}H]glucose. A low-intensity UV detector (340 nm) was used to monitor the fractions (1 mL/fraction). A 0.50-mL sample of vesicles with [^{3}H]glucose was applied to the column by a syringe connected to a three-way valve. The syringe was washed three times with 0.5 mL of solvent (water or buffer). The flow rate was 1 mL/min, the void volume was 11 mL, and the vesicles were recovered between 12 and 16 mL of elution. The free glucose was found between 22 and 40 mL of elution. Between the vesicle fractions and the free glucose the radioactivity was 5–10% of the glucose activity found in the vesicle fractions. Figure 1 shows a typical column profile for poly(α-MA) vesicles with [^{3}H]glucose.

Scintillation Counting. Aliquots (25–75 μL) of the sample to be measured were pipetted into 20-mL screw-cap glass vials. Scintillation solution (10-mL portions) (Kodak Ready-to-Use II 13277) was added,

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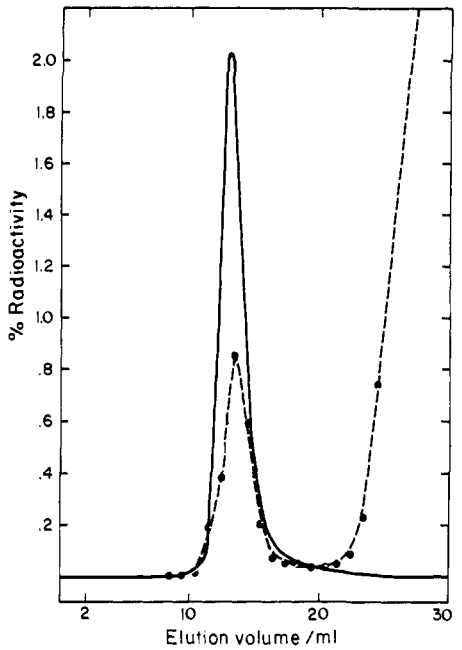

Figure 1. Column profile for the separation of vesicle [poly(α-MA) membranes] entrapped [^{3}H]glucose from free [^{3}H]glucose on a 1.6 × 40 cm Sephadex G 50 column, ●--●--●. Also shown is the apparent absorption at 340 nm due to light scattering of the vesicles, —.

and the vials were mixed thoroughly by hand. The sample vials were counted in a Packard Tricarb Model 3003 scintillation spectrometer. Column samples were counted for 1 min, and dialysis samples were counted for 10 min to give good statistics. Before the vesicles with [^{3}H]glucose were applied to the column, an aliquot was taken to assay the total radioactivity applied to the column. A 25-μL vesicle sample was diluted to 20 mL, and aliquots of 25, 50, and 75 μL were used for scintillation counting. The average value was taken as the total radioactivity of the vesicle sample.

Dialysis. After chromatography, 2.0-mL portions of the vesicle fractions (usually fractions 13 and 14 and 50 μL of fraction 15) were placed in a dialysis bag and dialyzed against 200 mL of water or buffer with stirring in a graduated cylinder. Aliquots (50 μL) were taken from the dialyzate, and the radioactivity was determined by scintillation counting at selected intervals. Upon completion of the dialysis, we obtained infinity values by opening the contents of the dialysis bag to the dialyzate. The infinity values agreed well with the original total activity in the sample. The total activity in the dialysis bag was calculated from the activity in the column fractions.

Glucose Permeability. A qualitative picture of the permeability of lipid membranes to glucose was obtained from the fraction of [^{3}H]glucose released from the dialysis bag vs. time. The permeability data were analyzed by the method of Johnson and Bangham.[1,22] This analysis assumes that the fast initial rate is due to the outer bilayer of the liposomes and that the permeability of the dialysis bag is fast and may be neglected compared to the bilayer permeability. The dialysis bag permeability was evaluated with free [^{3}H]glucose in the bag in the presence or absence of α-MA vesicles. The vesicles did not affect the dialysis rate of the free [^{3}H]glucose out of the bag. In both cases, the half-time of the radioactivity loss from the bag was about 50 min, which is significantly faster than with samples with the [^{3}H]glucose in the vesicles.

The analysis of Johnson and Bangham[1,22] yields

$$\ln\left[\frac{N_1^1 V_0}{V_1 + V_0} - N_0\right] = \ln N_1^1\left[\frac{V_0}{V_1 + V_0}\right] - k_1\frac{V_0 + V_1}{V_0}t \qquad (1)$$

where

$$k_1 = (A_1/V_1)P_1 \qquad (2)$$

A_1 = surface area of the membranes, V_1 = internal volume of the membranes, P_1 = permeability of the membrane, N_1^1 = counts in membranes at $t = 0$, V_0 = volume of the dialyzate, and N_0 = counts in dialyzate at $t = t^1$. A plot of eq 1 as a function of time gives a straight line whose slope, $k_1[(V_0 + V_1)/V_0]$, is related to the size and the permeability of the membranes. Evaluation of the vesicle membrane size by light scattering or electron microscopy allows the permeability P_1 to be calculated.

Samples for Inelastic Light Scattering. Prepared vesicle suspensions were filtered through polycarbonate nucleopore filters of 1.0 μm size, through which 100 mL of distilled water had been filtered, into 1-cm square quartz fluorescence cells. The cells had been cleaned with detergent and repeatedly rinsed with filtered water expelled from the cell with argon filtered through 0.02-μm nucleopore membranes. Before addition of initiator and subsequent incubation, the vesicle size and mean scattering intensity were measured by inelastic light scattering. The light-scattering photometer consists of a Spectra-Physics 50-mW helium–neon laser focused through the scattering cell. Scattered light was measured at a scattering angle of 90° through a solid angle of 10^{-4} steradians by focusing the scattered light onto a pinhole of ~0.1 mm radius placed in front of a Bendix Channeltron photomultiplier. The field of view was adjusted to just fit the 1-mm^2 photocathode area. The photocurrent was processed as a train of photon pulses, which were then processed digitally by photon-counting circuitry. The photon pulses were statistically analyzed by a Honeywell SAICOR Model 42 correlator, which gave a decaying correlation function computed and stored digitally in 100 channels, each corresponding to a (selectable) time duration of τ seconds. For these particular samples τ was usually 20 ms. The digital content of the correlator's 100 channels was transferred as 30-bit integers to a DEC 20–20 system computer for subsequent curve fitting and plotting.

Results and Discussion

Vesicle Formation and Polymerization. Two methacryloyl lipids and two butadiene lipids were used in this study. Aqueous suspensions of the polymerizable lipids were sonicated at 50 °C, above the lipid-phase transition, 40 and 28 °C for the α-MA and ω-MA samples, respectively, to yield opalescent dispersions. The aqueous dispersions of methacryloyl lipids were thermally polymerized after argon purging. After polymerization, a sample of each dispersion was freeze-dried, dissolved in $CDCl_3$, and examined by ^{1}H NMR. The disappearance of the vinyl protons indicates at least 90% reaction of the monomeric lipid. The butadiene lipid dispersions were polymerized by UV light. The chromophore of butadiene PC absorbs at 257 nm with ϵ 2.7 × 10^4. These data compare favorably with those of ethyl sorbate, which has a λ_{max} of 261 nm in hexane with ϵ 2.6 × 10^4,[23] and of a similar butadiene derivative, 1,2-bis(octadeca-2,4-dienoyl)-*sn*-glycero-3-phosphorylcholine (λ_{max} 261 nm in EtOH).[24] Irradiation under argon led to a loss of 98% of the sorbate absorption.

The dispersions of lipids effectively trap water-soluble compounds (Figure 1). The behavior of dispersions of the monomeric lipids on gel permeation chromatography is typical of lipid vesicles. Chromatography of the polymerized dispersions is similar to that of the monomeric vesicles. The retention time and percent entrapment of aqueous markers are not significantly affected by the polymerization. This shows that the entrapped volume of the vesicle is not greatly perturbed by polymerization.

Permeability. Membrane permeability of vesicular systems can be measured with any water-soluble marker. Useful detection methods based on fluorescence, enzymatic, redox, electron paramagnetic resonance, and radiochemical techniques are commonly used. [^{3}H]Glucose was chosen for this study because it does not interact with cationic membrane lipids and can be used at low ionic strength, a necessity because the cationic vesicles tend to aggregate at salt concentrations >20 mM.

Glucose is a convenient choice because it has a reasonable permeability through monomeric membranes; therefore, if polymerization of the membrane alters the permeability, the effect can be detected on a time scale of several hours rather than days. The permeability of glucose through phosphatidylcholine vesicles is ~10^{-10} cm/s, which is about 10^3 faster than monovalent cations (Na^+, K^+: 10^{-13} cm/s)[1,25] and is slower than urea (~10^{-5} cm/s) or water (~10^{-2} cm/s).

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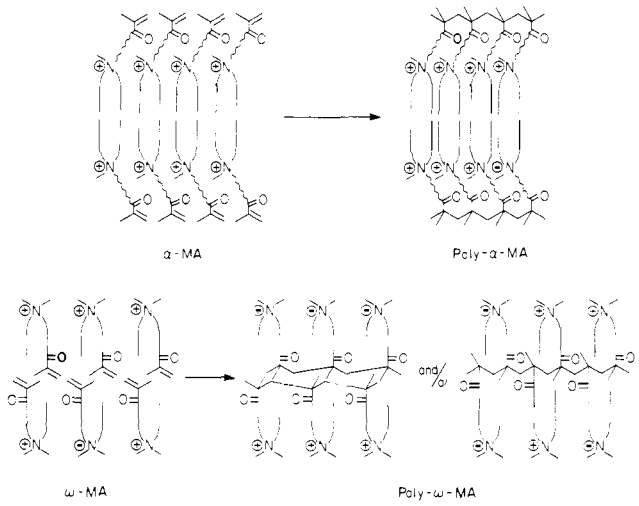

Figure 2. Schematic representation of α-MA and ω-MA lipids in a bilayer membrane before and after formation of poly(α-MA) and poly(ω-MA).

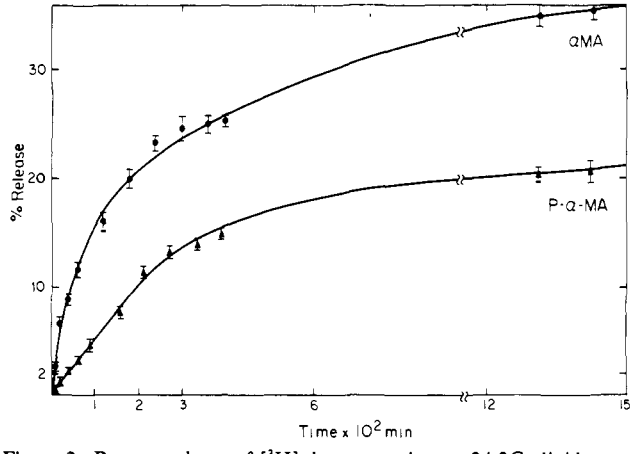

Figure 3. Percent release of [³H]glucose vs. time at 24 °C: lipid membranes of α-MA, ●—●; poly(α-MA), ▲—▲.

The methacryloyl lipids are both cationic and differ in the location of the polymerizable group. In α-MA, the methacrylamide is attached to the hydrophilic head group, and ω-MA has the methacrylate attached to one of the hydrophobic chains. The polymeric α-MA consists of a polymer chain near the aqueous interface of the lipid bilayer with the water. In Figure 2, the polymer chain is represented at the aqueous interface, but because of its hydrophobicity it may reside in the bilayer. Dorn has shown that poly(α-MA) and poly(ω-MA) display phase transitions at 33 and 10 °C, respectively; thus they still exhibit hydrophobic chain mobility.[26]

The permeability data show a fast initial rate followed by a slow approach to equilibrium for monomeric and polymeric α-MA. This behavior is probably due to the presence of multilamellar liposomes in the sample preparation. The faster initial [³H]glucose release is due to the population of unilamellar vesicles and the permeability through the outer bilayer of multilamellar liposomes. A typical permeability profile for glucose in monomeric and polymeric α-MA liposomes is shown in Figure 3. The same data are used in expression 3 to give the logarithmic plot shown in Figure 4. Also included for comparison is the leakage of glucose from the dialysis bag.

Polymeric butadiene lipid vesicles also show decreased membrane permeability after polymerization and usually do not show a phase transition below 100 °C.[24] Two butadiene lipids, butadiene taurine and butadiene PC, each of which has a sorbate in both hydrocarbon chains, were used for comparison with the methacryloyl lipids.

(26) Dorn, K., Doctoral Dissertation, University of Mainz, 1983.

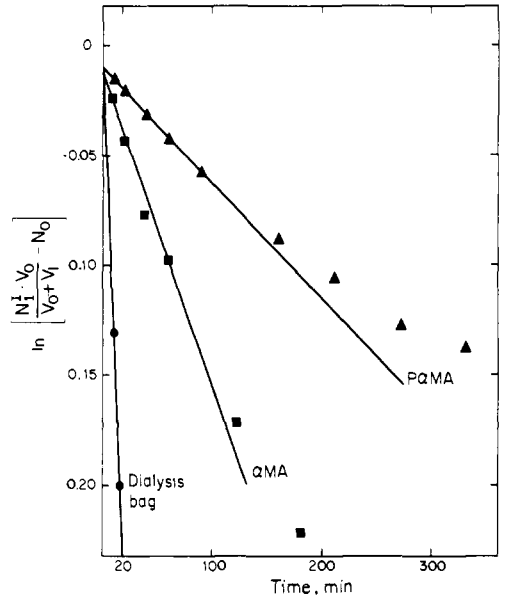

Figure 4. Semilogarithmic plot of the fraction $\ln [N_1^t V_0/(V_0 + V_1) - N_0]$ vs. time for [³H]glucose permeation through the dialysis bag, ●—●; membrane bilayers of α-MA, ■—■; poly(α-MA), ▲—▲.

Table I. Estimated Permeability of Monomeric and Polymeric Lipid Bilayer Membranes to [³H]Glucose at 24 °C

lipid	k_1 (10^{-4} s^{-1})	P^a (10^{-9} cm s^{-1})
α-MA monomer	2.22 ± 0.07	0.74
polymer	0.88 ± 0.01	0.29
ω-MA monomer	4.35 ± 0.31	0.72
polymer	2.34 ± 0.22	0.39
butadiene taurine monomer	3.45 ± 0.10	(1.15)
polymer	1.93 ± 0.05	(0.64)
butadiene PC monomer	3.83 ± 0.02	(1.29)
polymer	1.15 ± 0.05	(0.39)
DODAB	0.595 ± 0.048	0.20
dialysis bag	24.08 ± 0.26	

[a] Calculated on the basis of vesicles of 1000-Å radius for the α-MA membranes and of 500 Å for the ω-MA membranes as determined by inelastic light scattering. The values of P for butadiene taurine and butadiene PC were calculated assuming a 1000-Å radius for these membranes as well.

The slopes of the semilogarithmic plot (k_1) and permeability constant (P) are given in Table I for the monomeric and polymeric liposomes studied. The permeability constant was estimated from the sizes of monomeric and polymeric α-MA and ω-MA, which were determined by inelastic light scattering (see below). The radii of the α-MA liposomes range from 200 to 1000 Å, and those of the ω-MA liposomes vary from 200 to 500 Å. The larger values were used for the calculation of P in Table I. If we assume that the butadiene lipid vesicles are similar in size to α-MA, a preliminary estimate of permeability constants can be obtained.

Polymerization of α-MA was more reliable with the water-soluble initiator AAPH than with AIBN. Poly(α-MA) vesicles prepared with AAPH showed reduced glucose permeability compared with the monomeric α-MA vesicles, whereas reaction of α-MA vesicles with AIBN in some instances gave a product with glucose permeability similar to that of the monomeric α-MA vesicles. The NMR spectra of vesicle samples prepared with AAPH and AIBN did not show vinyl protons. These data suggest that a high-molecular-weight polymer of α-MA was more likely to be formed with the water-soluble initiator AAPH than with AIBN, and that under some conditions only short-chain oligomers were produced with AIBN. Polymerization of ω-MA was efficient with AIBN and gave high-molecular-weight polymer in the lipid bilayer with reduced permeability to glucose.

The molecular weight of poly(α-MA) and poly(ω-MA) formed in bilayer membranes was estimated by gel permeation chromatography.[27] The apparent number average ($\bar{M}_n$) for poly(α-MA)

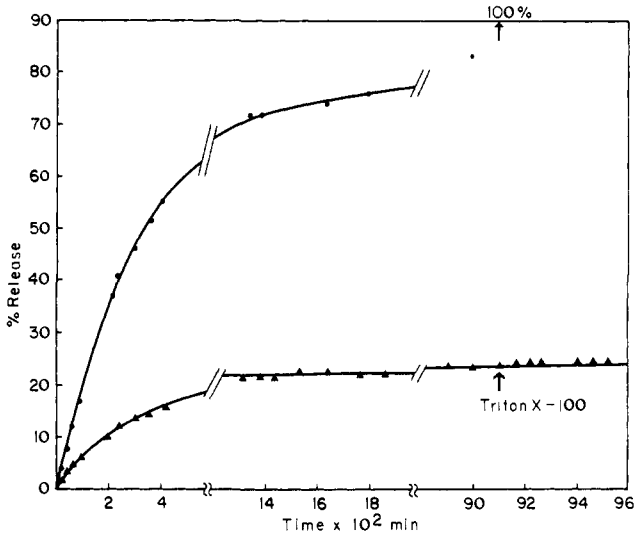

Figure 5. Percent release of [^{3}H]glucose vs. time at 24 °C for lipid membranes of butadiene PC, ●—●; poly(butadiene PC), ▲—▲. Both membranes were treated with Triton X-100 surfactant at 9100 min. The butadiene PC membranes released the remaining entrapped [^{3}H]glucose, whereas the poly(butadiene PC) membranes retained the entrapped [^{3}H]glucose after surfactant treatment.

was 3.5×10^5, and the weight average ($\bar{M}_w$) was 1.9×10^6. The corresponding values for poly(ω-MA) were 3.9×10^5 ($\bar{M}_n$) and 9.5×10^5 ($\bar{M}_w$). The values of $\bar{M}_n$ suggest that the average poly(α-MA) and poly(ω-MA) consisted of about 500 monomer units. These data coupled with the vesicle radii determined by light scattering yield an estimate of 20 to a few hundred polymer chains in a highly polymerized vesicle.[27]

In each case in Table I the permeability was reduced by the presence of polymer in the membrane vesicle. The reduction was a factor of 2.5 and 1.8 for α-MA and ω-MA, respectively. Regen et al. reported permeability of [^{14}C]sucrose in methacryloyl-substituted phosphatidylcholines before and after UV-light-induced polymerization.[18] Although initial rates of sucrose release and permeabilities were not reported, after 4 h the percent of the sucrose released was about twice as great for the monomeric vesicles as the polymerized vesicles. Their experiments showed a reduction in membrane permeability to uncharged water-soluble species similar to what we have observed. Further decreases in permeability may be possible by cross-linking the polymer chains in the membrane bilayer.

The butadiene lipids are cross-linkable by virtue of the reactive group in each acyl chain. Photolysis of the butadiene taurine sufficient to decrease the butadiene absorption to less than 5% of the initial value yielded a membrane vesicle that was about 50% as permeable to glucose as the monomeric vesicles. A similar experiment with butadiene PC vesicles resulted in 98% reduction of the diene absorption with a decrease in the initial glucose permeability to about 30% of that of the monomeric vesicles. Furthermore, after ~20% of the glucose escaped the polymerized vesicle preparation (Figure 5), no further glucose was lost even when the membranes were treated with Triton X-100 surfactant (0.1% final concentration). This suggests the possibility that the poly(butadiene PC) vesicles consist of two populations; in one the vesicles are effectively sealed to glucose permeation, and in the second population (~20%) there is sufficient residual unpolymerized butadiene to allow a slow leakage of glucose.

The results with butadiene PC show that appropriately cross-linked polymer networks in the membrane bilayer can significantly reduce the permeability of water-soluble uncharged species, e.g., glucose, through lipid vesicles. These data extend the previous observations of Schupp and Ringsdorf, who reported that poly(butadiene PC) vesicles are impermeable to the charged species 6-carboxyfluorescein.[6,24,28] Thus, uncharged as well as ionized water-soluble molecules can be effectively entrapped in selected polymeric vesicles for at least several days.

The greater reduction of glucose permeability in poly(butadiene PC) compared with poly(butadiene taurine) may be due to the higher photochemical conversion of the butadienes in the former lipid. Further evaluation is necessary to test this hypothesis. In butadiene PC the α- and β-chain butadienes are not likely to react with one another, since the two fatty acid chains of phosphatidylcholines extend unequal distances into membrane bilayers.[7,29] Therefore, poly(butadiene PC) could be highly cross-linked. On the other hand, the fatty acid chains in the butadiene taurine extend equal distances into the bilayer, and the two butadiene groups are likely to react with one another as well as butadienes in neighboring lipid molecules. The polymer chain is expected to have only occasional cross-links and thus be less effective at sealing the vesicle.

Determination of Vesicle Size by Inelastic Light Scattering. The Brownian motion of vesicles in suspension results in a fluctuating intensity of scattered light. Statistical analysis of the intensity fluctuation shows that for a monodisperse suspension the fluctuations decay exponentially with a time constant inversely proportional to the vesicle diffusivity D. If one assumes that the diffusivity is related to vesicle radius a according to the Stokes–Einstein relation, $D = kT/6\pi\eta a$, where k is Boltzmann's constant, T is the absolute temperature, and η is the suspension medium viscosity, then measurement of the relaxation time yields the vesicle radius. In practice, suspensions are sufficiently polydisperse that the observed fluctuation relaxation is not simply exponential so that no single relaxation time describes the relaxation for both short and long duration. One empirical procedure to estimate the extent of polydispersity is to compare the "apparent" relaxation time obtained from a "best" nonlinear fit of the observed relaxation to a single exponential function for shorter and shorter durations. For very narrow size distribution, the apparent diffusivity decreases linearly with increasing duration. As the polydispersity increases, this linear dependence vanishes and quantitative measure of polydispersity becomes much more difficult, requiring high precision of the measured relaxation as well as complex mathematical fitting methods. However, as a first estimate, the vesicle size is best approximated by the smallest size measured, as larger particles contribute so strongly to the fluctuations that they totally dominate the measured size if present in fractions greater than a few tenths of a percent. Therefore, in the following reported vesicle sizes the dominant size is assumed to be that obtained for durations of 200 μs, and the extent of dispersity indicated by the relative increase in apparent size as duration increases to 2 ms.

Vesicles of α-MA in the absence of initiator are extremely stable, as indicated by the lack of significant increase in mean apparent size; even the distribution of sizes as indicated by the set of variously fitted durations remains nearly constant for several days. Nonfractionated α-MA vesicles (~200–1000 Å in radius) showed larger mean apparent size than fractionated α-MA vesicles (~200–300 Å in radius). This probably indicates simply the dominance of a small amount of larger particles and not the predominant size. Since very little material was pelletted by centrifugation, it can be assumed that the predominant vesicle radius was ~200–300 Å. The larger particles are colloidally stable, showing no significant size increase even in the presence of initiator, during the first day after incubation. This would be consistent with a low percentage of vesicles cross-linked to one another, which would have little effect on the mean size of large particles. A single filtered sample was divided into three portions and filtered into cells without further dilution (before incubation). The fitted mean apparent sizes showed no difference with extent of polymerization (time of incubation) although there was a slight decrease in size upon polymerization, whereas there was no change

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in size of the incubated "monomeric" vesicles.

The vesicular suspensions of ω-MA were not colloidally stable, whereas the poly(ω-MA) suspensions were stable. Within hours of centrifugation the mean size was significantly increasing, and the suspensions appeared flocculent after incubation overnight. The initial sizes indicate a slightly smaller size than the α-MA vesicles, in the 170-250-Å range. After incubation, the nonpolymerized vesicles could not be fitted to a single experimental relaxation function. This generally indicates a bimodal distribution, i.e., two major sizes, with few intermediately sized particles. Again, the smaller of the two sizes is usually predominant.

Polymerization of the vesicle membrane alters the predominant radius of the vesicles prepared by sonication to ~300-400 Å. Although larger particles are present, they do not represent a significant fraction of material as little material pelletted upon centrifugation, which yielded the small predominant apparent mean-sized vesicles.

Conclusions

Thermally initiated polymerization of bilayer membranes of α-MA and ω-MA gave polymers with retention of the vesicle structure. The mean size distribution of the colloidal suspension was not significantly altered by the polymerization. The membranes still sequestered [^{3}H]glucose, and the permeability of the bilayer membrane decreased to about 0.5 of that of the nonpolymerized membranes. The number-average molecular weights estimated for poly(α-MA) and poly(ω-MA) suggest that the average polymer chain consists of 500 units.[27] These data coupled with the radii of the vesicles show that there are 20 to a few hundred polymer chains per vesicle of poly(α-MA) or poly(ω-MA).

The observed moderate reduction in permeability on polymerization is probably a consequence of the large number of polymer chains per vesicle. Differential scanning calorimetry of these membranes shows that the phase transition has been shifted only a few degrees; thus the lipid chain mobility in both poly(α-MA) and poly(ω-MA) is not greatly reduced. Membrane permeability may be further reduced by cross-linking the polymer chains, either with appropriately designed lipids, which contain multiple reactive groups, or by the addition of membrane-soluble cross-linking agents to lipids such as ω-MA.

The first approach is exemplified by poly(butadiene PC) vesicles, which showed a significant reduction in glucose permeability. These vesicles successfully encapsulated glucose for several days and were not disrupted by the addition of surfactant. Photopolymerization of butadiene taurine vesicles only moderately reduced membrane permeability. The difference in permeability properties of polymerized membranes of each of these suggests that effective reduction in membrane permeability is more likely with lipid molecules that contain reactive groups arranged to enhance intermolecular rather than intramolecular reaction.

Finally, caution must be exercised in describing the materials formed upon polymerization of lipids in vesicle structures. In many instances the vesicles will consist of several polymer chains, which enhance the stability of the vesicle to various reagents and conditions, without necessarily producing a major change in membrane permeability. However, polymerization of appropriately designed lipids, e.g., butadiene PC, can yield vesicles with enhanced stability and significantly reduced permeability to nonionic water-soluble species.

Registry No. α-MA, 81571-93-9; poly(α-MA), 87279-13-8; ω-MA, 87279-14-9; poly(ω-MA), 87279-15-0; butadiene PC, 88589-84-8; poly(butadiene PC), 88589-85-9; butadiene taurine, 88589-82-6; poly(butadiene taurine), 88589-83-7; D-glucose, 50-99-7; dioctadecylamine, 112-99-2; acrylonitrile, 107-13-1; 3-(dioctadecylamino)propionitrile, 28288-18-8; *N*,*N*-dioctadecyl-1,3-propanediamine, 15337-59-4; methacryloyl chloride, 920-46-7; *N*-[3-(dioctadecylamino)propyl]methacrylamide, 76282-14-9; 12-hydroxydodecanoic acid methacrylate, 63439-30-5; 6-(dimethylamino)-1-hexanol, 1862-07-3; 12-methacryloyloxydodecanoic acid *N*,*N*-dimethylhexanamine ester, 88589-86-0; octadecyl bromide, 112-89-0; triethyl 4-phosphonocrotonate, 10236-14-3; dodecylaldehyde, 112-54-9; (*E*,*E*)-2,4-hexadecadienoic acid ethyl ester, 59404-47-6; 2,4-hexadecadienoic acid, 59404-48-7; *N*,*N*-bis(2-hydroxyethyl)-2-sulfoethylamine, 10191-18-1; 2,4-hexadecadienoic anhydride, 88589-87-1.

Electrochemically Switched Cation Binding in Nitrobenzene-Substituted, Nitrogen-Pivot Lariat Ethers

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Abstract: *N*-2-Nitrobenzylmonoaza-15-crown-5 (**2**) and *N*-4-Nitrobenzylmonoaza-15-crown-5 (**4**), representatives of a new class of N-pivot lariat ethers bearing reducible nitroaromatic side arms, have been prepared and examined by cyclic voltammetric techniques. When the nitro group is sterically disposed to permit interaction with the ring-bound cation (**2**), intramolecular ion pairing occurs. This is not the case when the side arm is remote from the macroring (**4**) nor when the side arm is detached. A thermochemical cycle is used to determine that the binding constant (K_s) enhancement in MeCN solution is 25000-fold upon electrochemical switching.

We have previously reported the synthesis of macrocyclic polyether compounds having donor-group-bearing side arms attached at both carbon[1] and nitrogen pivot points.[2] These lariat ethers exhibit a variety of interesting properties, but in general, the N-pivot compounds have proved more versatile as enhanced cation binders than the C-pivot structures. We believe that this is due, at least in part, to the geometrical requirements of cation binding. In the N-pivot structures, cation binding by the macroring involves the nitrogen lone pair and forces the side arm into a more or less perpendicular position appropriate for a secondary

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