Polymerized–Depolymerized Vesicles. Reversible Thiol–Disulfide-Based Phosphatidylcholine Membranes[1]

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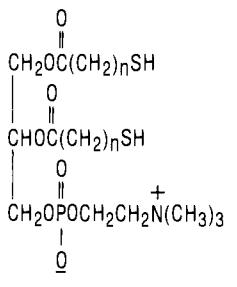
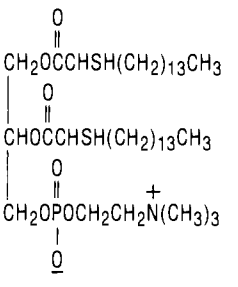
Abstract: The syntheses and characterizations of 1,2-bis(11-mercaptoundecanoyl)-*sn*-glycero-3-phosphocholine (**1a**), 1,2-bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (**1b**), and 1,2-bis(2-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (**2**) are reported. Each of these phospholipids has been used to construct vesicles that can be oxidatively polymerized by using hydrogen peroxide as well as reductively depolymerized, via dithiothreitol or 1-octanethiol. On the basis of the residual thiol content and the measured liposomal diameters, well-sonicated and polymerized vesicles of **1a**, **1b**, and **2** are estimated to contain an average of 80, 350, and 100 polymer chains, respectively; the number average degree of polymerization is estimated to be 25, 17, and 20, respectively. Oxidation and reduction efficiency, entrapment and permeability properties, phase-transition behavior, and stability features for each vesicle network are reported. The possible relevance of such polymerizable–depolymerizable vesicles to membrane modeling and drug delivery is briefly discussed.

Phospholipid bilayer vesicles (liposomes) bear a close structural relationship to naturally occuring membranes.[4-6] The fact that they can be assembled in a pure state as well as in the presence of *selected* components (e.g., proteins, steroids, etc.) has made them particularly attractive for model studies. In addition, liposomes are now receiving considerable attention as adjuvants for the controlled delivery of drugs and as devices for photochemical solar energy conversion.[5-8] In this paper we introduce a new and unique class of phospholipid vesicles which can be polymerized ("switched on") and depolymerized ("switched off"), via a thiol–disulfide redox cycle.[9-14] These membranes are based on the thiol-bearing phosphatidylcholine molecules **1a**, **1b**, and **2** whose syntheses are also described herein.

Our principal motivation for the construction of polymerized–depolymerized liposomal networks has been threefold. First, reversibly polymerized vesicles should constitute highly flexible biomembrane models. In the polymerized state (the "on position"), lateral diffusion within the bilayer should be significantly reduced or eliminated; in the depolymerized mode (the "off position"), lateral diffusion should be restored. One area where such membranes might prove particularly useful is in the field of immunochemistry. The lateral mobility and distribution of membrane antigens/haptens is believed to play an important role in the immune response.[15] In principle, polymerized (or partially polymerized)–depolymerized vesicles could be used to fine-tune the lateral motion of haptens, to hold them in place, and to release them at will. Furthermore, the ability to depolymerize a vesicle network would allow one to take apart and recover key membrane components.[16]



1a, n = 10
b, n = 15



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A second reason for preparing reversibly polymerized vesicles relates to their potential biodegradability and utility as drug carriers. Polymerized phospholipid vesicles have been suggested for use in drug delivery.[10,14] It is significant to note, however, that with the exception of one polyamide-based liposome,[13b] all polymerized vesicles that have been reported thus far possess nonbiodegradable all-carbon backbones.[10-14] Polymerized vesicles that are susceptible to depolymerization in vivo would clearly be more desirable. The fact that the disulfide moiety is common to many biopolymers suggests that disulfide-based polymerized vesicles may be fully biodegradable.

Finally, the concept of *an ordered network of monomers capable of reversible polymerization* is, to the best of our knowledge, fundamentally new. The construction of such a unique molecular assembly, in and of itself, represents a worthy synthetic challenge.

In principle, phospholipids bearing two or more thiol groups should be capable of yielding polymerizable–depolymerizable vesicle networks. The essential criterion for polymerization is that the oxidation process proceed via *interlipid* coupling. On the basis of the preferred conformation of phospholipids in the bilayer state, we reasoned that there should be a natural predisposition toward interlipid coupling for vesicles comprised of **1a**, **1b**, or **2**. Low-angle X-ray diffraction and NMR analysis indicate that for saturated phosphatidylcholines, the glycerol backbone is approximately perpendicular to the plane of the bilayer,[17,18] where the two fatty

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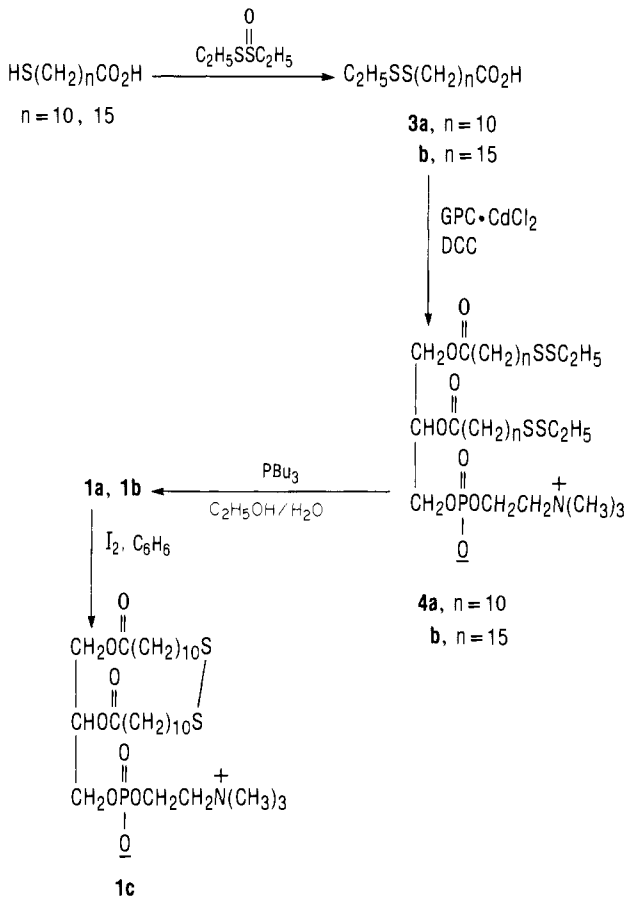
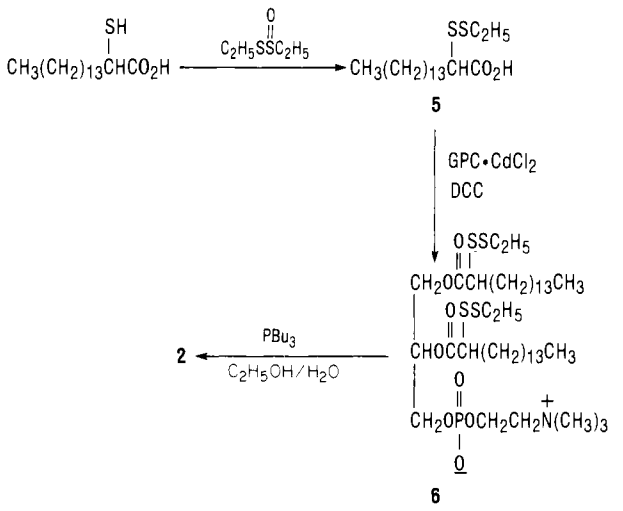
Scheme I



Scheme II



acid chains extend unequally into the membrane. If a thiol group were positioned at identical carbon atoms in each of the C-1 and C-2 chains, the C-1 thiol moiety should be in closer proximity to the center of the bilayer; the C-2 thiol would lie closer to the inner and outer surface of the membrane. This segregation of thiol groups should result in preferred interlipid coupling.[19] The fact that disulfide bonds can be reduced to the thiol state under mild conditions further suggests that retention of a vesicle structure should be possible. In principle, therefore, a redox cycle should provide the necessary means for polymerizing and depolymerizing vesicles comprised of **1a**, **1b**, or **2**.

Results and Discussion

Lipid Synthesis. The synthetic routes used for the preparation of **1a**, **1b**, and **2** were unexceptional in nature and are outlined in Schemes I and II. Treatment of 11-mercaptoundecanoic acid with ethyl ethanethiosulfinate in chloroform produced an 80% isolated yield of 11-(ethyldithio)undecanoic acid (**3a**); subsequent esterification with *sn*-glycero-3-phosphocholine–$CdCl_2$ (GPC-$CdCl_2$) furnished a 91% isolated yield of 1,2-bis[11-(ethyldithio)undecanoyl]-*sn*-glycero-3-phosphocholine (**4a**). Reduction of **4a** with tri-*n*-butylphosphine in C_2H_5OH–H_2O (1/1) afforded a 95% isolated yield of **1a**. Lipids **1b** and **2** were prepared by using similar synthetic methods.

Vesicle Formation. Vesicles derived from **1a**, **1b**, and **2** were prepared by (a) coating the lipid onto the walls of a round-bottomed flask (chloroform evaporation), (b) dispersing the lipid into a 10 mM borate buffer (pH 8.5) containing 140 mM NaCl and 2 mM NaN_3 (vortex mixing), and (c) irradiating the dispersion with ultrasound at 50 °C under a nitrogen atmosphere to constant turbidity. Thin-layer chromatography plus thiol analysis indicated that no lipid decomposition occurred during sonication. Opalescent to optically clear aqueous dispersions were obtained in all cases. Vesicles formed from **1b** exhibited the poorest stability, precipitating on standing after a few hours at room temperature. Gel filtration of dispersions of **1a** and **2** using a Sepharose 6B column resulted in a 96% and 90% vesicle recovery in the void volume, respectively (phosphorus analysis). Vesicles of **1b** proved to be too unstable to survive similar gel filtration. The greater inherent stability of **1a** relative to **1b** is surprising in view of the shortness of its aliphatic chains. We have, at present, no explanation to account for this behavior.

Table I. Oxidatively Polymerized Phospholipid Vesicles

initial monomer composition[a]	thiol content remaining,[b] %	$\bar{X}_n$	$\bar{M}_n$
1a	4	25	16450
1b	6	17	13300
2	5	20	15960

[a] Oxidation was carried out at pH 8.5 using ca. 1.5 mg of phospholipid/milliliter and 20 equiv of 30% H_2O_2 for 4 h at 40 °C. In all cases the change in turbidity after oxidation was less than 7%. [b] The thiol/phosphorus ratio, determined for dispersions of **1a**, **1b**, and **2** before oxidation, ranged between 1.6 and 1.8. The percentage of remaining thiol groups was determined by using the phosphorus analysis as an internal reference.

Oxidative Vesicle Polymerization. Oxidative polymerization of all vesicle dispersions was carried out by treatment with excess H_2O_2 at 40 °C for 4 h. Qualitative thin-layer chromatography showed in each case a single spot at the origin. In order to establish that *interlipid* oxidative coupling had occurred, an authentic sample of the cyclic monomer **1c** (Scheme I) was synthesized via oxidation of **1a** with iodine in benzene under high-dilution conditions. The monomeric nature of this product was confirmed by fast atom bombardment mass spectrometry which showed a parent ion at MH^+ m/z 656; no evidence for dimer formation was found. Migration of **1c** on silica gel was very similar to that of its thiol precursor [R_f = 0.26 (**1a**); R_f = 0.30 (**1c**); silica, $CHCl_3/CH_3OH/H_2O$ (65/25/4)]; qualitative analysis (Ellman test) also confirmed the absence of thiol groups. While no attempt has been made to prepare the corresponding cyclic monomers of **1b** and **2**, the close structural similarity of these lipids to **1a** strongly suggests that the cyclic analogues should exhibit similar R_f values. The lack of migration of oxidized **1b** and **2** (aqueous dispersions) from the origin on silica gel is, therefore, taken as positive evidence for dominant interlipid coupling.

The turbidity of all dispersions, measured as the apparent absorbance at 400 nm, changed by less than 7% after oxidation. Thiol analysis showed that extensive oxidation had occurred (Table I). Analysis of polymerized **1a** (obtained from a vesicle dispersion after lyophilization) in CD_3OD by ^1H NMR (250 MHz) indicated a ratio of CH_2S/CH_2CO = 1.0; no signals were observed in the

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Table II. Reductive Depolymerization of Phospholipid Vesicles

initial monomer composition	reducing agent	regenerated monomer[a]	polymeric lipid, %
1a	DTT	63	26
	OT	67	7
1b	DTT	66	17
	OT	85	5
2	DTT	93	9
	OT	81	21

[a] Reductive depolymerization was carried out by adding (a) 200 equiv of solid dithiothreitol (DTT) or (b) 200 equiv of 1-octanethiol (OT) to the vesicle dispersion (0.75 mg of lipid in 0.5 mL of water), followed by brief shaking and heating (50 °C, 1.5 h); turbidity changes in all cases were less than 7%. Depolymerization in 10 mM borate buffer (pH 8.5) and in pure water gave comparable results. Increasing the reaction time did not increase the yield of monomeric phospholipid. Phosphorus analysis was made for the following products: R_f(**1a**) = 0.26 ± 0.03; R_f(**1b**) = 0.21 ± 0.03; R_f(**2**) = 0.25 ± 0.03. In each case, $CHCl_3$–CH_3OH–H_2O (65/25/4) was used as the solvent system. A control experiment indicated that ca. 90% of the phosphorus placed on a silica gel plate (in the form of oxidatively polymerized vesicles of **2**) was detected by using procedures described in the Experimental Section. Numbers reported are corrected values.

region expected for methylene protons α to sulfoxide or sulfone groups (i.e., δ = 2.7–3.2). Overoxidation of the disulfide moiety, therefore, does not appear to proceed to any significant degree under the reaction conditions used.

Gel filtration (Sepharose 6B) of polymerized aqueous dispersions of **1a** and **2** afforded a vesicle recovery of 90% and 95%, respectively, in the void volume of the column. Interestingly, in the polymerized state, vesicles derived from **1b** exhibited substantially improved stability and could be recovered in the void volume (85%) of a Sepharose 6B column. Electron microscopic examination of polymerized dispersions of **1a**, **1b**, and **2** confirmed the presence of closed spheres. The average diameter of polymerized vesicles derived from **1a** and **2** was ca. 200 Å; polymerized vesicles formed from **1b** had an average diameter of ca. 300 Å.

Degree of Polymerization within the Bilayer. If it is assumed that intramolecular coupling is negligible within these vesicle bilayers, reasonable estimates of the number average degree of polymerization, $\bar{X}_n$, and the number average molecular weight, $\bar{M}_n$, can be made by using eq 1 and 2;[20] here, p = the extent of conversion (as determined by the remaining thiol content) and M_0 is the molecular weight of the lipid monomer. Thus, in the

$$\bar{X}_n = 1/(1-p) \quad (1)$$

$$\bar{M}_n = M_0\bar{X}_n \quad (2)$$

oxidized state, vesicles of **1a**, **1b**, and **2** contain an average of ca. 25, 17, and 20 lipids per polymer chain, respectively (Table I). Based on these values, a crude estimate of the average number of polymer chains per vesicle is also possible. If it is assumed that (1) all the vesicles are unilamellar, (2) the bilayer thickness is ca. 50 Å,[21] and (3) each lipid occupies an area of 70 Å^2, then the calculated number of lipids per average vesicle of **1a**, **1b**, and **2** is ca. 2000, 6000, and 2000, respectively. This estimate implies that to a first approximation, polymerized vesicles of **1a**, **1b**, and **2** bear an average of 80, 350, and 100 polymer chains, respectively.

Reductive Vesicle Depolymerization. Treatment of a polymerized vesicle dispersion of **1a** with 200 equiv of dithiothreitol (DTT) for 1.5 h at 50 °C led to ca. a 63% yield of regenerated monomer [quantitative thin-layer chromatography (Table II)]; 26% of the lipid remained at the origin. The ^{1}H NMR and IR spectra of **1a**, isolated from the DTT-treated mixture, were identical with that found for the starting lipid. When the more hydrophobic thiol reducing agent, 1-octanethiol (OT), was used, the percentage of regenerated monomer was 67%; only 7% of the lipid remained at the origin. Qualitative inspection of the thin-layer chromatographic plates indicated the presence of lipid components lying in the region between R_f = 0.0 and 0.26. these products are presumed to be oligomeric in nature and have not been analyzed either quantitatively or qualitatively. Analogous results were obtained for the depolymerization of polymerized vesicles of **1b** (Table II). The lower quantity of lipid measured at the origin for polymerized dispersions of **1a** and **1b** using OT as a reducing agent is a likely result of more efficient disulfide reduction due to a high local concentration of the lipophilic thiol in the hydrocarbon core of the bilayer. Interestingly, under similar reactions conditions, polymerized vesicles of **2** were reduced to a greater extent with DTT than with OT (Table II). In this case, the greater efficacy of DTT can be accounted for in terms of (1) the greater accessibility of the disulfide groups to the aqueous phase, where a higher effective concentration of DTT should be present, and (2) the greater reducing power of the dithiol.[22] Changes in apparent turbidity in all cases were minimal (less than 7%) after treatment with either DTT or OT. Gel filtration of depolymerized vesicles of **1a** and **2** (using DTT) indicated an 85% and 92% recovery in the void volume of a Sepharose 6B column. Regenerated vesicles of **1b** proved to be too unstable for gel filtration.

Table III. Entrapment of and Permeability Toward Sucrose

vesicle composition[a]	entrapment, %	capture vol,[b] M^{-1}	retention, % 2 h	retention, % 4 h
1a	0.06	0.09	50	40
P-**1a**	0.07	0.09	58	33
P-**1b**	0.10	0.60	61	13
2	0.74	1.25	72	56
P-**2**	0.72	1.14	75	58

[a] Dispersions were formed from 10 mg of phospholipid in 1.0 mL of 10 mM borate buffer (pH 8.4) containing 10 μCi of [^{14}C] sucrose. Gel filtration (Sephadex G-50-150) and dialysis (against 50 mL of pure water at 23 °C) were similar to those previously described.[10] The prefix P refers to the polymerized state. [b] Capture volume (L/mol) represents the percent entrapment × 100, divided by the lipid concentration in the vesicle state (as determined by phosphorus analysis of the void volume after gel filtration). Incubation of nonpolymerized vesicles of **1a** and **2** with 10 μCi of [^{14}C]sucrose for 30 min indicated an *apparent* capture volume (adsorption) of 0.031 and 0.080 M^{-1}, respectively.

Entrapment and Permeability. In order to measure the entrapment and permeability properties of these phospholipid membranes in the polymerized and nonpolymerized state, [^{14}C] sucrose was entrapped within the aqueous vesicle interiors by using standard procedures.[10] Table III summarizes the results obtained. Nonpolymerized and polymerized vesicles of **1a** exhibited relatively small capture volumes (expressed as liters/mole) and moderate permeability. Vesicles of **1b** showed a significantly larger capture volume in the polymerized state and similar permeability properties. Because of the inability of nonpolymerized vesicles of **1b** to survive gel filtration, corresponding entrapment and permeability measurements could not be made. Phospholipid vesicles of **2** in the polymeric and monomeric form showed the highest capture volume and the lowest permeability. Within experimental error, polymerization of this membrane (as well as vesicle membranes of **1a**) had no significant influence on its permeability. This insensitivity of membrane permeability toward polymerization is in contrast to poly(methacrylate) analogues previously reported[10] and may be accounted for by the relatively low degree of polymerization obtained.

Phase-Transition Behavior. Changes in absorbance at 400 nm as a function of temperature were used to monitor phase-transition behavior in the vesicular state.[23] As shown in Figure 1, a well-defined phase transition at ca. 22 °C was evident for non-

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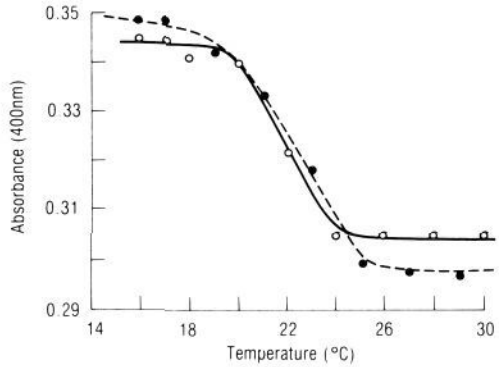

Figure 1. Plot of apparent absorbance (turbidity) at 400 nm as a function of temperature for nonpolymerized vesicles of **2**: (●) heating cycle; (○) cooling cycle.

polymerized vesicles of **2**; within the limits of detectability, subsequent polymerization did not alter the phase-transition temperature. For vesicles of **1b** (in the polymerized state) and **1a** (polymerized and nonpolymerized state), no apparent phase transitions were observed between 10 and 60 °C. The well-defined transition for **2** is not surprising in view of the fact that the polymer backbone is positioned near the head group. Nearly all the hydrocarbon bilayer is, therefore, free to undergo cooperative disordering via the introduction of gauche rotational isomers.[24] For polymerized vesicles of **1a** and **1b**, the backbone (running through the core of the bilayer) undoubtedly restricts such structural reorganization. Similar differences in phase-transition behavior have previously been noted in polymerized methacrylate-based phosphatidylcholine vesicles.[25]

Vesicle Stability. On standing at 25 °C, nonpolymerized vesicles of **1a** remain stable for ca. 48 h; after 72 h, the dispersion shows a very substantial increase in turbidity. Oxidatively polymerized analogues show no significant change in turbidity up to 6 days at 25 °C. Beyond this period, however, there is substantial precipitation. Improvement in the shelf life upon polymerization is also evident for vesicles formed from **1b** and **2**. Here, constant turbidity is extended to 48 and 72 h, from 4 to 10 h, respectively. Polymerization, however, *does not* significantly improve the stability of these vesicles toward lysis using 0.6% sodium dodecylsulfate (SDS); i.e., in all cases, the turbidity is decreased by ca. 50%. Qualitatively, therefore, disulfide-based polymerized vesicles of the type described herein do not exhibit the extraordinary stability which has been found in polymerized vesicles derived from 1,2-bis[12-(methacryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine. The latter show no evidence of disruption upon addition of up to 7% SDS.[26] High vesicle stability is likely to be found only with *cross*-linked polymerized vesicle membranes.[26]

Polymerization Across the Bilayer? One intriguing structural question that relates to polymerized vesicles is whether or not coupling occurs *across* the bilayer. The likelihood of such an occurrence should increase as the polymerizable groups are placed closer to the terminus of the hydrocarbon tails, especially the C-1 chain. Freeze-fracture electron micrographs of polymerized vesicles derived from an isocyano amphiphile have been used as qualitative evidence for polymerization across the bilayer.[27] Here, the assumptions are that (1) in the nonpolymerized state, convex and concave three-dimensional half-ball patterns result from fracture planes which run through the middle of the bilayer and (2) in the polymerized state, the fracture planes cut through the bilayer and water (ice) compartments (cross-fracture), leading to two-dimensional circles. In preliminary freeze-fracture experiments performed with 1-palmitoyl-2-[12-methacryloyloxy)-dodecanoyl]-*sn*-glycero-3-phosphocholine, we have made similar observations: polymerized samples produced two-dimensional circles, and nonpolymerized analogues yielded concave and convex half-balls.[28] Freeze-fracture electron micrographs of polymerized vesicles of **1a** reveal three-dimensional half-balls (Figure 2). While this result suggests that polymerization is largely confined within the inner and outer monolayers, we cannot exclude the possibility that there is substantial polymerization across the bilayer but that the degree of polymerization is too low to produce cross-fracture patterns.

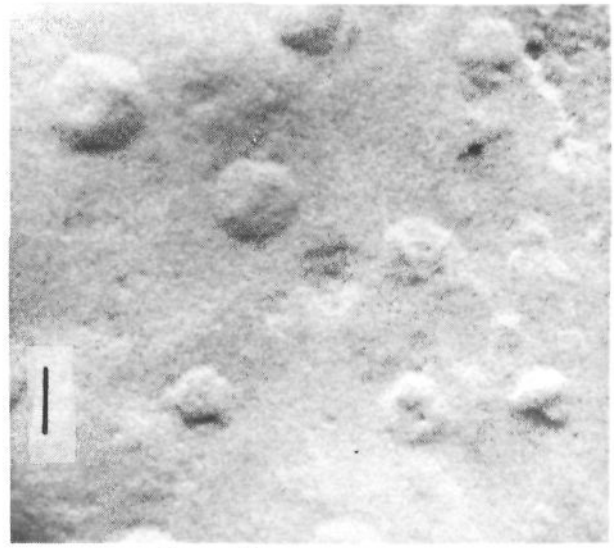

Figure 2. Freeze-fracture electron micrograph of polymerized vesicles of **1a**. Bar represents 1000 Å.

Finally, preliminary photobleaching recovery experiments, using 3,3′-dioctadecyloxacarbocyanine perchlorate as a probe, indicate that lateral diffusion within vesicle membranes of **1a** is reduced by approximately 1 order of magnitude upon polymerization, to a value of 2×10^{-9} cm^2/s.[29,30] Studies which are now in progress are aimed at developing effective means for cross-linking polymerized liposomes of **1a**, **1b**, and **2** in order to improve their stability and to further reduce lateral diffusion within the membrane. Results of these efforts will be reported in due course.

Experimental Section

General Methods. Unless stated otherwise, all chemicals and reagents were obtained commercially and used without further purification. Deionized water was distilled twice from $KMnO_4$. *sn*-Glycero-3-phosphorylcholine (GPC) was prepared from egg lecithin as previously described[10] and converted into its $CdCl_2$ complex (GPC-$CdCl_2$) by using procedures similar to those previously described.[31] [^{14}C]Sucrose (360 mCi/mmol, 20% ethanol solution) was obtained from ICN Laboratories. 11-Bromoundecanoic acid and 2-bromohexadecanoic acid (Aldrich Chemical Co.) were converted into 11-mercaptoundecanoic acid[32] and 2-mercaptohexadecanoic acid,[34] respectively, by using procedures similar to those previously described in the literature; 16-bromohexadecanoic acid was similarly converted into its thiol derivative.[33,34] Ethyl ethanethiosulfinate was prepared by oxidation of the corresponding disulfide.[35] 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent) was purchased from Aldrich Chemical Co. and used directly. Dialysis experiments were carried out by using Spectropor No. 6 dry membrane tubing (cylindrical diameter of 14.6 mm; MW cutoff 6000–8000) obtained from Spectrum Medical Industries. Vesicle dispersions were prepared either in pure

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water or in a 10 mM borate buffer (pH 8.5) containing 140 mM NaCl and 2 mM NaN_3. Chloroform used in depositing the phospholipids onto the walls of the glass tubes was HPLC grade (Fischer Scientific); chloroform used for chromatography was reagent grade (Aldrich Chemical Co.). Turbidity was determined by measuring the apparent absorbance at 400 nm. ^1H NMR, IR, and UV spectra were recorded on a Varian EM 360L or a Bruker WM-250 MHz, Beckman Acculab 7, and Bausch & Lomb Spectronic 2000 spectrometers, respectively. Elemental analyses were performed by Midwest Microlab, Indianapolis, IN. Mass spectra were performed at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, NE. Phase-transition measurements were carried out by using a Perkin-Elmer 320 spectrophotometer coupled with a digital temperature controller. Chromatographic separations were carried out by using precoated Merck 0.25-mm silica gel 60 TLC plates (with fluorescent indicator) and Merck 70-230 ASTM silica gel with the following eluting solvents mixtures: (A) 1% CH_3OH–$CHCl_3$; (B) $CHCl_3$–CH_3OH–H_2O (4/5/1); (C) $CHCl_3$–CH_3OH–H_2O (65/25/4); (D) $CHCl_3$–CH_3OH (1/1). Unless stated otherwise, detection on TLC plates was made using iodine vapor or a UV lamp. Sonications were performed by using a Heat Systems Model W-375 R bath-type sonicator. Vortex mixing was carried out by using a VWR Scientific mixer (Model K-550 G). Specific procedures used for electron microscopy and entrapment of and permeability toward [^{14}C]sucrose were similar to those previously described;[10] electron micrographs recorded using negative staining (2% uranyl acetate) were taken by using a Philips 400 STEM microscope.

11-(Ethyldithio)undecanoic Acid (3a). A mixture of 11-mercaptoundecanoic acid[32] (1.40 g, 6.42 mmol), ethyl ethanethiosulfinate[35] (1.18 g, 8.55 mmol), and triethylamine (0.72 mL, 5.2 mmol) in 16 mL of chloroform was stirred at room temperature for 24 h. Evaporation of solvent under reduced pressure followed by chromatographic purification of the residue on a silica gel column (2.5 × 40 cm), eluting first with $CHCl_3$ and then solvent A, furnished 1.43 g (80%) of 11-(ethyldithio)-undecanoic acid: R_f = 0.52 (5% CH_3OH in $CHCl_3$); IR (nujol) $\nu_{C=O}$ 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (br s and t, 19 H, CH_2 and CH_3CH_2S), 2.33 (t, 2 H, CH_2CO), 2.45–2.9 (m, 4 H, CH_2S), 11.7 (s, 1 H, CO_2H).

1,2-Bis(11-(ethyldithio)undecanoyl)-*sn*-glycero-3-phosphocholine (4a). To a mixture of GPC-$CdCl_2$ (89.6 mg, 0.196 mmol), 11-(ethyldithio)-undecanoic acid (222 mg, 0.80 mmol), and 4-(dimethylamino)pyridine (48.0 mg, 0.40 mmol) dissolved in 2 mL of freshly distilled $CHCl_3$ was added 165 mg (0.80 mmol) of dicyclohexylcarbodiimide. The resulting mixture was then stirred for 48 h at room temperature in the dark. After removal of solvent in vacuo, the residue was dissolved in a minimum volume of solvent B and then applied to a 1.2 × 7 cm column of AG MP-50 resin (50–100 mesh, hydrogen form). Elution with 30 mL of solvent B followed by solvent evaporation and chromatographic purification using a 1 × 20 cm silica gel column, eluting with solvents D and C, respectively, afforded 139 mg (91%) of **4a**; having an R_f = 0.22, solvent C: IR $\nu_{C=O}$ 1720 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1050, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (br s and t, 38 H, CH_3CH_2S and CH_2), 2.28 (m, 4 H, CH_2CO), 2.5–2.9 (m, 8 H, CH_2S), 3.36 (s, 9 H, $N(CH_3)_3$), 3.65–4.5(m, 8 H, CH_2O and CH_2N), 5.15 (m, 1 H, CHO).

1,2-Bis(11-mercaptoundecanoyl)-*sn*-glycero-3-phosphocholine (1a). To a solution of 87 mg (0.112 mmol) of **4a**, dissolved in 1 mL of ethanol plus 1 mL of water, was added 0.11 mL (0.45 mmol) of tri-*n*-butylphosphine. After the mixture was stirred at room temperature for 11 h (in the dark), the solvent was then evaporated under reduced pressure and the residue purified by column chromatography on silica gel (1 × 20 cm), eluting with solvent D and C, successively. The phospholipid product (70 mg, 95%) showed a single spot by TLC, having R_f = 0.26 (solvent C): IR (neat) $\nu_{C=O}$ 1730 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1060, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (br s, 32 H, CH_2), 2.0–2.75 (m, 8 H, $HSCH_2$ and CH_2CO), 3.35 (s, 9 H, $N(CH_3)_3$), 3.65–4.5 (m, 8 H, CH_2O and CH_2N), 5.15 (m, 1 H, CHO). Anal. Calcd for $C_{30}H_{60}O_8NS_2P$: N, 2.13; S, 9.75; P, 4.71. Found: N, 1.92; S, 8.38; P, 4.87.

16-(Ethyldithio)hexadecanoic Acid (3b). Procedures used for the synthesis of **3b** were similar to those described above for the preparation of **3a**. The isolated yield of product based on 16-mercaptohexadecanoic acid[33,34] was 64%: R_f = 0.52 (5% CH_3OH in $CHCl_3$); IR (nujol) $\nu_{C=O}$ 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (br s and t, 29 H, CH_2 and CH_3CH_2), 2.4 (t, 2 H, CH_2CO), 2.5–2.9 (m, 4 H, CH_2S), 10.9 (br s, 1 H, CO_2H).

1,2-Bis(16-(ethyldithio)hexadecanoyl)-*sn*-glycero-3-phosphocholine (4b). Procedures used were similar to those described above for the preparation of **4a**. The yield of the phospholipid, based on the starting carboxylic acid, was 91%: R_f = 0.30, solvent C; IR (nujol) $\nu_{C=O}$ 1740 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1050, and 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–1.4 (br s and t, 58 H, CH_2 and CH_3CH_2S), 2.3 (t, 4 H, CH_2CO), 2.5–2.8 (m, 8 H, CH_2S), 3.35 (s, 9 H, $N(CH_3)_3$), 5.15 (m, 1 H, CHO).

1,2-Bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (1b). Procedures used were similar to those used for the preparation of **1a**, described above; the isolated yield of **1b** was 98%: R_f = 0.30, solvent C; IR (nujol) $\nu_{C=O}$ 1730 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1060, and 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (br s, 52 H, CH_2), 2.1–2.7 (m, 10 H, CH_2CO, CH_2S, SH), 3.4 (s, 9 H, $N(CH_3)_3$), 3.7–4.3 (m, 8 H, CH_2O and CH_2N), 5.15 (m, 1 H, CHO). Anal. Calcd for $C_{40}H_{80}O_8PNS_2$: N, 1.76; S, 8.03; P, 3.88. Found: N, 1.95; S, 6.35; P, 4.08.

2-(Ethyldithio)hexadecanoic Acid (5). Procedures used for the synthesis of **5** were similar to those described above for the preparation of **3a**. The isolated yield of product, based on 2-mercaptohexadecanoic acid, was 72%: R_f = 0.63 (5% CH_3OH in $CHCl_3$); IR (nujol) $\nu_{C=O}$ 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H, CH_2CH_3), 1.2–1.3 (br s, 29 H, CH_2 and CH_3CH_2S), 2.4–2.5 (q, 2 H, CH_3CH_2S), 3.2–3.3 (t, 1 H, CHS), 10.5 (s, 1 H, CO_2H).

1,2-Bis(2-(diethyldithio)hexadecanoyl)-*sn*-glycero-3-phosphocholine (6). Procedures used were similar to those used for the preparation of **1a** described above; the isolated yield was 82%: R_f = 0.30, solvent C; IR (nujol) $\nu_{C=O}$ 1725 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1060, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3CH_2), 1.2–1.3 (br s and t, 58 H, CH_2 and CH_3CH_2S), 2.4–2.5 (q, 4 H, CH_3CH_2S), 3.2–3.4 (br s, 11 H, $N(CH_3)_3$ and CHS), 3.7–4.5 (m, 8 H, CH_2O and CH_2N), 5.15 (m, 1 H, CHO).

1,2-Bis(2-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (2). Procedures used were similar to those used for the preparation of **1a** described above; the isolated yield of **2** was 76%; R_f = 0.30, solvent C; IR (nujol) $\nu_{C=O}$ 1725 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1060, and 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3CH_2), 1.2–1.3 (br s and t, 52 H, CH_2 and CH_3CH_2S), 2.2 (br s, 2 H, SH), 3.2–3.4 (br s, 11 H, $N(CH_3)_3$ and CHS), 3.7–4.5 (m, 8 H, CH_2O and CH_2N), 5.2 (m, 1 H, CHO). Anal. Calcd for $C_{40}H_{80}O_8PNS_2$: N, 1.76; S, 8.03; P, 3.88. Found: N, 1.71; S, 6.88; P, 4.22.

Cyclized Monomer 1c. Phospholipid **1a** (20 mg, 0.03 mmol) was dissolved in 40 mL of benzene containing 8.54 μL (0.06 mmol) of triethylamine. A solution of 7.84 mg (0.03 mmol) of iodine dissolved in 20 mL of benzene was then added dropwise over a period of 1 h. The combined mixture was stirred for 5 h at room temperature and then washed successively with 5% sodium thiosulfate and distilled water. The organic layer was dried over sodium sulfate and concentrated, and the crude product was purified by chromatography (silica gel) using a gradient of $CHCl_3$ and solvents D and C to give 5.0 mg (25%) of **1c**: R_f = 0.25, solvent D; IR (nujol) $\nu_{C=O}$ 1725 cm^{-1}; IR $\nu_{N(CH_3)_3}$ 1090, 1065, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (br s, 32 H, CH_2), 2.3 (m, 4 H, $CH_2C{=}O$), 2.5–2.7 (m, 4 H, CH_2S), 3.4 (s, 9 H, $N(CH_3)_3$), 3.7–4.5 (m, 8 H, CH_2O and CH_2N^+), 5.2 (m, 1 H, CHO); fast atom bombardment, MH^+ m/z 656.

Vesicle Preparation. Typically, 2.0 mg (0.003 mmol) of **1a**, dissolved in 0.1 mL of HPLC-grade chloroform, was placed in a $^1/_2$ in. × 4 in. test tube and the solvent slowly evaporated with the aid of a stream of nitrogen. The tube was then evacuated (18 h, 22 °C (1.0 mm)), sealed with No-Air stopper, and flushed with a stream of nitrogen. A borate buffer (2 mL) containing 140 mM NaCl and 2 mM NaN_3 (pH 8.5) was added to this tube via a syringe, and the tube was then placed in a water bath (50 °C) for 10 min. A multilamellar dispersion was then formed by vigorous vortex mixing for 2 min. Subsequent sonication in a bath-type sonicator at 50 °C for 1 h produced a clear stable dispersion whose optical density remained constant upon further sonication. Thin-layer chromatography on silica gel (solvent C) indicated that no lipid decomposition occurred; i.e., a single spot was observed, having an R_f = 0.22.

Thiol Analysis. A 0.2 M Tris buffer (pH 8.2) was prepared from tris(hydroxymethylamino)methane and its hydrochloride salt and subsequently diluted with an equal volume of absolute ethanol (containing 1% EDTA). To 10 mL of the resulting buffer was added 40 mg (0.101 mmol) of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB reagent solution). For thiol analysis, 0.040 mL of a given vesicle dispersion was added to 1.5 mL of the above buffer solution (containing ethanol and EDTA) plus 0.1 mL of the DTNB reagent solution and the absorbance at 412 nm measured after 20 min at room temperature.[36]

Phosphorus Analysis. Procedures used for the determination of phosphorus were similar to those previously described.[37] Analysis of dispersions derived from nonpolymerized **1a**, **1b**, and **2** for both thiol and phosphorus content yielded a molar ratio of sulfur to phosphorus ranging from 1.6 to 1.8.

Qualitative Thin-Layer Chromatography. Vesicle dispersions (before and after polymerization) were spotted on a TLC plate, dried under a stream of nitrogen, and developed with solvent C. Products were detected either by iodine vapor or by spraying the plate with 0.1% DTNB in the

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Tris buffer described (containing ethanol and EDTA).

Quantitative Thin-Layer Chromatography. After qualitative thin-layer chromatographic analysis was made (iodine detection), silica gel containing adsorbed lipid was removed from appropriate zones and analyzed directly for phosphorus content. Procedures used were similar to those previously described,[37] except a larger volume of magnesium nitrate solution (ca. 0.20 mL) was employed, and the derivatized mixture was filtered to remove silica gel prior to UV analysis.

Vesicle Polymerization. Typically, 7 μL of 30% H_2O_2 (20 equiv) was added to a 1.0-mL dispersion of **1a** containing 2.0 mg of lipid, and the resulting dispersion was heated under a nitrogen atmosphere for 3 h at 40 °C. Thin-layer chromatography showed a single spot at the origin (iodine). The dispersion was then dialyzed against 100 mL of doubly distilled water for 18 h at room temperature in order to remove excess hydrogen peroxide. Quantitative analysis for thiol content indicated that ca. 5% of the thiol groups remained.

Vesicle Depolymerization. Typically, a 1.0 mL of polymerized dispersion of **1a** (2.0 mg of lipid) that had been dialyzed to remove excess hydrogen peroxide was purged with a stream of nitrogen and mixed with 60 mg (0.388 mmol) of dithiothreitol. The resulting dispersion was purged with nitrogen for 5 min and heated in a water bath for 1.5 h at 50 °C. Qualitative analysis by TLC indicated substantial regeneration of **1a**; R_f = 0.22, solvent C, positive thiol test. Quantitative phosphorus analysis revealed a 63% yield of regenerated **1a**.

Freeze-Fracture Electron Microscopy. Samples of sonicated and polymerized dispersions of **1a** (20 mg/mL) were frozen in liquid nitrogen, fractured with etching by using a Balzer apparatus, and replicated with Pt/C by using standard procedures.[38] Glycerol (5%) was added to prevent freeze damage. Electron micrographs of freeze-fractured samples were obtained by using a Philips 201 instrument.

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Registry No. 1a, 87050-11-1; **1b**, 93404-44-5; **1c**, 93404-45-6; **2**, 93404-46-7; **3a**, 87050-15-5; **3b**, 93404-47-8; **4a**, 87050-14-4; **4b**, 93404-48-9; **5**, 93404-49-0; **6**, 93404-50-3; GPC, 28319-77-9; dithreitol, 3483-12-3; ethyl ethanethiosulfinate, 18542-39-7; hydrogen peroxide, 7722-84-1; 11-mercaptoundecanoic acid, 71310-21-9; 1-octanethiol, 111-88-6.

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Electron-Relay Chain Mechanism in the Sensitized Photoisomerization of Stilbazole Salts in Aqueous Anionic Micelles

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Abstract: Cis to trans isomerization of *N*-methyl-4-(β-styryl)pyridinium halide (4-MSPX, X = I or Cl) via electron-transfer sensitization by $Ru(bpy)_3^{2+}$ has been studied for some micellar systems under the illumination of 468 ± 5 nm light. Efficiencies for the isomerization ($\phi_{c\rightarrow t}$) were markedly enhanced on addition of anionic surfactans; e.g., the quantum yields in the presence of sodium dodecylsulfate (SDS) reached the maximum value of 64 ± 2, which is about 100-fold as much as those without SDS. The value is comparable to the aggregation number, N_A, of the SDS micelle, involving the pyridinium ions equal to SDS molecules. Similar agreement between N_A and $\phi_{c\rightarrow t}$ values was observed with micelles of other anionic surfactants $CH_3(CH_2)_nOSO_3Na$ (n = 9, 11, 13). It is postulated that an electron-relay chain mechanism is operative on the anionic micellar surface for the sensitized isomerization of pyridinium ions attached electrostatically. The line-width measurements of ^{23}Na NMR indicated that 4-MSP ion effectively substitutes the Na^+ ion adsorbed on SDS micelles. An overwhelming high adsorptivity of 4-MSP ion on anionic micelles over Na^+ ion was also evidenced by effects of various added salts in the reaction mixture.

An efficient electron transfer between donor D* and acceptor A (eq 1) in micellar systems is the subject of recent interest. The

$$D^* + A \rightarrow D^+ + A^- \quad (1)$$

$$D^+ + A^- \rightarrow D + A \quad (2)$$

heterogeneous electrostatic field formed by surfactant molecules is considered to retard the back transfer of an electron once generated (eq 2), enhancing the efficiency of net charge separation.[1] Thus, much attention has been focused on redox reactions utilizing anionic micellar systems in relation to solar energy storage.[2] Interaction of a hydrated electron with surfactant-solubilized benzene, for example, showed significant micellar effects;[3] the electron addition to the benzene molecule was retarded by sodium dodecyl sulfate (SDS) but enhanced by the cetyltrimethylammonium bromide (CTAB) micelle. The two opposite effects suggest that the penetration of e_{aq}^- into the micelle interior of solubilized benzene is hindered by the negatively charged SDS micellar surface but enhanced by cationic CTAB micelle.[4]

As an alternative effect of micellar systems, anionic micelles are expected to force cationic substrates to aggregate on its negatively charged surface. There are few studies forcused on the reactions of substrates adsorbed on micellar surfaces. Electron

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