Cross-Linking Polymerization in Two-Dimensional Assemblies: Effect of the Reactive Group Site

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ABSTRACT: The cross-linking polymerization of hydrated amphiphiles in monolayers, bilayers, and nonlamellar phases, i.e., bicontinuous cubic and the inverted hexagonal phases, is an effective method to modify their properties. Polymerization of monomeric amphiphiles in an assembly proceeds in a linear or cross-linking manner depending on the number and location of polymerizable groups per monomer. Polymerization of hydrated lipids with reactive groups in each hydrophobic tail yields cross-linked polymers. Sisson et al. (1996) examined the cross-linking of bilayers as a function of the mole fraction of bis-substituted lipids (bis-SorbPC), where the reactive groups were located at the end of the lipid tails. The onset of cross-linking was determined by changes in lipid lateral diffusion, bilayer vesicle stability, and polymer solubility. These data indicated that a substantial mole fraction (0.30 ± 0.05) of the bis-substituted lipid was necessary for bilayer cross-linking. Analysis of the cross-linking and competing reactions suggested that the location of the reactive group, i.e., reaction site, in the amphiphile and therefore within the bilayer assembly influences the cross-linking efficiency. To assess this possibility, the cross-linking of dienoyl-substituted phospholipids, (*E*,*E*)-DenPC, where the reactive diene is located near the glycerol backbone of the lipid, was compared with SorbPC. The cross-linking of (*E*,*E*)-DenPC was found to be substantially more efficient than that of SorbPC. It is proposed that this effect is partly a consequence of the relative probability of macrocyclization and cross-linking reactions.

Introduction

The cross-linking polymerization of amphiphiles in monolayers, bilayers (lamellar), and nonlamellar phases, i.e., bicontinuous cubic and the inverted hexagonal phases, is an effective method to modify their properties.[1] Linear and cross-linked amphiphilic assemblies exhibit significant differences in physical properties, e.g., permeability,[2−4] chemical stability,[5−7] solubility,[5,7,8] lateral diffusion of components,[9] among others. Unlike monomers in isotropic media, the motions of hydrated amphiphilic monomers in assemblies are limited by the two-dimensional fluid of at least tens of thousands of amphiphiles. The hydrophilic headgroups are exposed to water, and the hydrophobic tails are aggregated to minimize water contact. In certain hydrated lipid phases the amphiphiles rapidly diffuse within the assembly, which affords ample opportunity for monomers to react with the propagating chain end.[9,10] In these cases the assembly is highly ordered yet sufficiently dynamic for efficient chain polymerizations.

Polymerization of monomeric amphiphiles in an assembly proceeds in a linear or cross-linking manner depending on the number and location of polymerizable groups per monomer.[7,11] Thus, for example, polymerization of bilayers composed of lipids containing a single reactive moiety in either of the hydrophobic tails or associated with the hydrophilic headgroup (monosubstituted lipids) yields linear polymers. Polymerization of lipids with reactive groups in each hydrophobic tail generally yields cross-linked polymers. Sisson et al. (1996) examined the cross-linking of bilayers as a function of the mole fraction of bis-substituted lipids.[7] The onset of cross-linking of both sorbyl (SorbPC) and acryloyl (AcrylPC) lipids was determined by changes in lipid lateral diffusion, bilayer vesicle stability, and polymer solubility (see Chart 1). In each monomeric lipid the reactive group(s) are located at the hydrophobic end of the lipid tails. Each method indicated that a substantial mole fraction (0.30 ± 0.05) of the bis-substituted lipid was necessary to cross-link bilayer membranes.

The study by Sisson et al. provided the initial insight into the nature of cross-linking in the constrained environment of organized assemblies and how it differs from isotropic polymerizations.[7] Significant differences between bilayer and solution or bulk polymerizations were previously revealed through systematic investigations of radical-initiated polymerizations in bilayers composed of acryloyl-, methacryloyl-, and sorbyl-substituted lipids.[12−14] At high conversions the polymer chains are likely to be terminated by reaction with initiator fragments, i.e., primary radical termination. Relatively large degrees of polymerizations, X_n, were observed for the radical polymerizations of acryloyl-, methacryloyl-, and sorbyl-substituted lipids in bilayers. Differences in reactivity of the propagating radical are also reflected in the size of the polymers obtained from the polymerization of bilayers of AcrylPC and SorbPC.[12,14] However, these experimentally observed differences do not account for the significant inefficiency in bilayer cross-linking of these amphiphiles. An analysis of the cross-linking and competing processes suggests that the location of the reactive group, i.e., reaction site, in the amphiphile and therefore within the bilayer assembly could influence the cross-linking efficiency.[15] To assess this possibility, we now compare and contrast the cross-linking of dienoyl-substituted lipids, (*E*,*E*)-DenPC, where the reactive diene is located near the glycerol backbone of the lipid, with SorbPC, where the reactive group is at the end of the lipid tail.

Results and Discussion

Lipid Synthesis. Both the mono- and bis-substituted dienoyl lipids were prepared from the dienoyl (Den) fatty acid **4** (Scheme 1), which was synthesized from the

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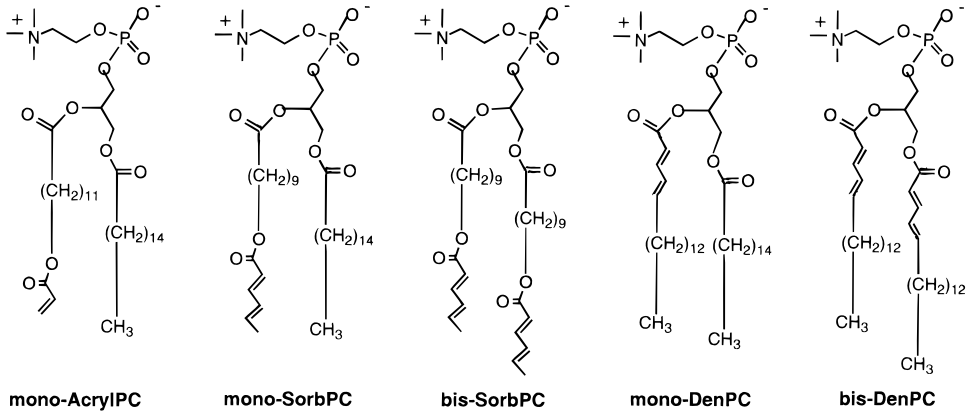
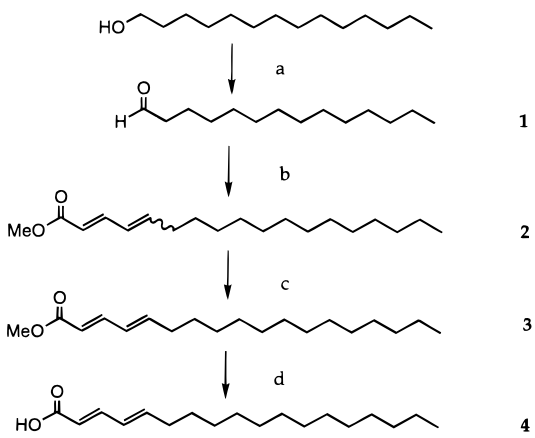
Chart 1



Scheme 1[a]



[a] (a) PDC, CH_2Cl_2; (b) NaH, trimethyl phosphonocrotonate, THF; (c) urea inclusion; (d) KOH, MeOH.

commercially available 1-tetradecanol. The alcohol was oxidized to the corresponding aldehyde **1** using pyridinium dichromate in CH_2Cl_2. The Wittig−Horner reaction of **1** and trimethyl 4-phosphonocrotonate gave a mixture of (*E*,*E*)- and (*E*,*Z*)-methyl dienoate (**2**). The (*E*,*E*)-Den acid **4** was obtained by basic hydrolysis of the (*E*,*E*)-ester (**3**) after its separation from its (*E*,*Z*)-isomer by urea inclusion.[16] Den acid **4** was used to acylate both glycerophosphorylcholine and 1-palmitoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine to obtain bis-(*E*,*E*)-Den$PC_{18,18}$ and mono-(*E*,*E*)-Den$PC_{16,18}$, respectively. The mono-(*E*,*E*)-DenPC bears the reactive group on the *sn*-2 chain.

Surfactant Solubilization of Vesicles. The previous studies by Sisson et al. demonstrated that cross-linked lipid vesicles are stable in the presence of excess surfactant, whereas unpolymerized or linearly polymerized vesicles are dissolved by surfactant.[7] At concentrations above the cmc a surfactant partitions into the lipid bilayer and eventually forms mixed micelles of surfactant and lipid.[17] The same process occurs with polymerized vesicles. However, cross-linked vesicles are not solubilized by added surfactant. If the vesicles are not cross-linked, they will be destabilized and form mixed micelles of poly(lipid) and surfactant.[7] Because mixed micelles of lipid and surfactants, such as Triton X-100, are usually spherical and less than 10 nm in diameter, they can be readily distinguished from lipid vesicles (ca. 100 nm diameter) by light scattering. If the poly(lipid) chains are not too long, then mixed micelles of the poly(lipid) and Triton X-100 are also small and readily distinguished from vesicles.[7] However, as a linear poly(lipid) increases in length, the mixed micelle increases in size and may change from spherical to rodlike. Consequently, it is desirable to use polymerization conditions that yield poly(lipids) of only moderate length in order to effectively use light scattering to differentiate between cross-linked and non-cross-linked vesicles.

Large unilamellar vesicles (LUV) composed of different mole fractions of mono- and bis-(*E*,*E*)-DenPC—100/0, 90/10, 85/15, 80/20, 70/30, and 0/100—in Milli-Q water were prepared by standard extrusion protocols.[18] The mean diameter of the LUV was determined by QELS to be 120 ± 10 nm. Differential scanning calorimetry was used to determine the main phase transition temperature, T_m, for each lipid. The T_m, i.e., the transition temperature between the solidlike bilayer phase and the higher temperature liquid crystalline phase, was 26.1 °C for mono-(*E*,*E*)-DenPC and 20.2 °C for bis-(*E*,*E*)-DenPC prepared for these studies. After preparation, the LUV were incubated at 60 °C for the polymerization. At this temperature, both zwitterionic lipids are in the liquid crystalline phase, and the various mixtures used in this study are expected to be random distributions of the two lipids.

The polymerization of each LUV sample composed of mono- and bis-(*E*,*E*)-DenPC was initiated by an equimolar mixture of potassium persulfate and sodium bisulfite at 60 °C.[19] The progress of the polymerization was monitored by changes in the monomer absorption at 260 nm (Figure 1). After 90% conversion, usually 18 h, the sample was cooled to room temperature and examined by quasi-elastic light scattering (QELS).[20] The degree of polymerization, X_n, which was determined by the methods previously described,[14] was controlled by selection of a [M]/[I] ratio of 5. Under these conditions the poly(lipid) formed from LUV of mono-(*E*,*E*)-DenPC are short enough to be incorporated into mixed micelles when treated with Triton X-100. The apparent diameter of these micelles is 10 nm (Figure 2). The stability of polymerized LUV to TX-100 is dependent on the initial composition of the LUV. Figure 2 shows the mean diameter of the samples as a function of the molar ratio of [Triton X-100]/[lipid]. The light scattering intensity of polymerized mono-(*E*,*E*)-DenPC LUV is substantially decreased upon addition of 2 equiv of Triton X-100. Further addition of Triton X-100 appears to have completely lysed the vesicles. The polymerized LUV composed of a 9/1 molar ratio of mono-(*E*,*E*)-DenPC and bis-(*E*,*E*)-DenPC shows a similar sensitivity to the addition of Triton X-100. In contrast to these observations, polymerized LUV comprised of 15 mol % or more

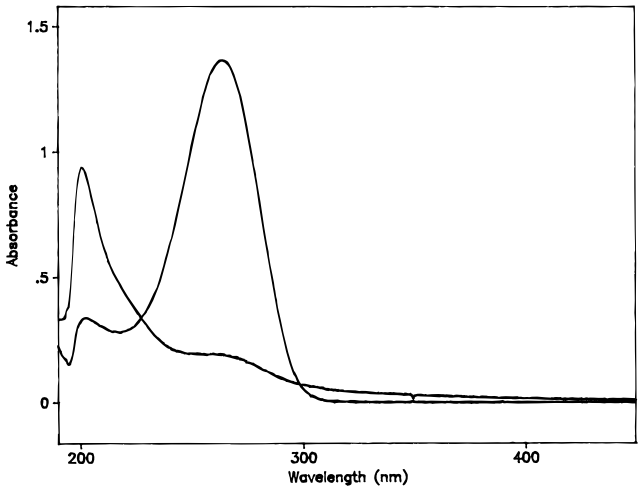


Figure 1. Absorption spectra of methanol extractions of liposomes composed of mono-(*E*,*E*)-DenPC before (λ_{max} 260 nm) and after polymerization as described in the Experimental Section.

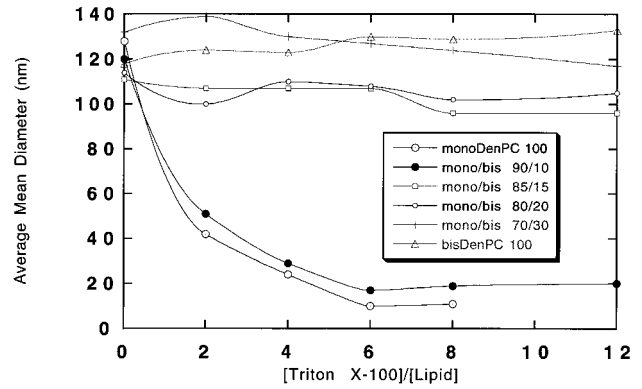


Figure 2. Representative experiment showing the average mean diameter (nm) of polymerized liposomes composed of the indicated molar ratios of mono-(*E*,*E*)-DenPC and bis-(*E*,*E*)-DenPC as a function of added equivalents of the surfactant Triton X-100.

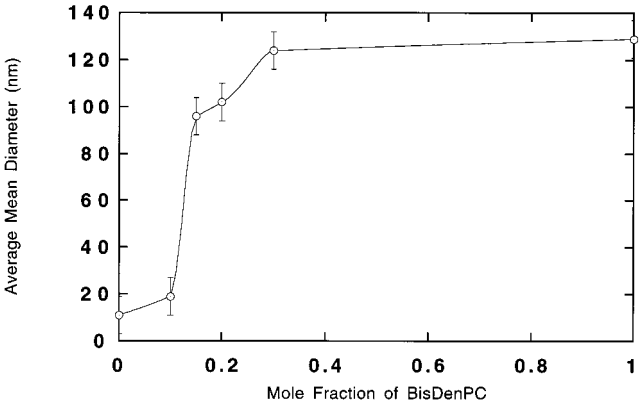


Figure 3. Average mean diameter (nm) of polymerized liposomes plus 8 equiv of Triton X-100 as a function of the mole fraction of bis-(*E*,*E*)-DenPC in mono-(*E*,*E*)-DenPC/bis-(*E*,*E*)-DenPC liposomes.

bis-(*E*,*E*)-DenPC are essentially unchanged in size by the addition of up to 12 equiv of Triton X-100.

The mean diameter of the polymerized LUV after addition of 8 equiv of Triton X-100 is plotted as a function of mole fraction of bis-(*E*,*E*)-DenPC in the bilayer at the time of the polymerization (Figure 3). These data show that the polymerized LUV sensitivity

Figure 4. Schematic representation of a small portion of half a bilayer of mono-SorbPC and bis-SorbPC where the reactive groups in the *sn*-1 chains of the bis-SorbPC react to form a link between poly(SorbPC) chains (a) and where the competitive macrocyclization of the bis-SorbPC yields a linear polymer chain segment (b).

to surfactant lysis is dramatically different if the initial mole fraction of bis-(*E*,*E*)-DenPC is 0.10 (soluble) as opposed to 0.15 (insoluble). This suggests that at high conversion to polymer the LUV will be cross-linked if the initial mole fraction of bis-(*E*,*E*)-DenPC is greater than 0.10 yet less than or equal to 0.15.

General Discussion

Our previous studies found effective cross-linking of hydrated lipid bilayer membranes of AcrylPC and SorbPC requires a high (0.30 ± 0.05) mole fraction of the respective bis-substituted lipid.[7,15] This contrasts with the normal situtation in isotropic media where quite low mole fractions of the bis-monomers are necessary for cross-linking. Inefficient cross-linking could be due to low conversion of monomers to polymer, significant differences in chemical reactivity of the monomers, and/or small degrees of polymerization. However, each of these appears unlikely to be a major contributor to the inefficient cross-linking, because the polymerizations were taken to high conversion; the relative reactivity of mono- and bis-SorbPC (as well as mono- and bis-AcrylPC) should be quite similar because the reactive group is the same in each monomer. Moreover, although the degrees of polymerization of mono-AcrylPC and mono-SorbPC differed, the cross-linking efficiency of each mono and bis pair of monomers was similar. Consequently, other explanations for the observed inefficiency in cross-linking of chain terminal substituted monomeric lipids were considered.

The high mole fraction of the bis-substituted lipid necessary for cross-linking could be due to the conformation of the lipids in the bilayer. The glycerol backbone of phospholipids in the L_β phase, i.e., the sample temperature is less than the T_m, is perpendicular to the plane of the bilayer as represented in Chart 1.[21,22] Therefore, when each lipid tail has the same length, the *sn*-1 chain penetrates deeper into the bilayer than the *sn*-2 chain. At temperatures above the T_m the motion of the lipid tails are more dynamic, especially for the portion of the lipid tail distal from the lipid headgroup. Both molecular simulations of PC bilayers and experimentally determined NMR order parameters of the individual carbons along the lipid chain indicate that the distal portion of the lipid chains exhibit considerably more disorder at temperatures above the T_m than below it.[23−25]

The reactive groups on each lipid tail of a polymerizable lipid, such as bis-SorbPC, are positionally inequivalent even though they have the same chemical composition. This suggests that bis-SorbPC could function as an AB type cross-linker (Figure 4a) that can

react with the mono-SorbPC primarily through the respective *sn*-2 chains. Under these circumstances a cross-link between polymer chains occurs when a reactive group on the *sn*-1 chain of a bis-SorbPC reacts with a similar group in a neighboring chain. Dimerization of *sn*-1 chains of bis-lipids provides a mechanism to cross-link the linear polymer chains formed by reaction at the *sn*-2 chains. Such a link between polymer chains may not always occur as shown in Figure 4a. It may be possible for the reactive group on the *sn*-1 chain of bis-SorbPC to react with mono-SorbPC to form a longer bridge between polymer chains. Because a 100 nm diameter liposome is composed of ca. 8×10^4 lipids, high conversion to mono-PC to polymers with a degree of polymerization of ca. 10^2 yields at least hundreds of polymer chains. If each bis-lipid in the chain efficiently formed a link with a bis-lipid on a neighboring chain, then only a few mole percent of the lipids would need to be bis-substituted. However, nearly one in three of the SorbPC lipids must be bis-substituted to achieve cross-linking. This suggests that most of the bis-lipids fail to create links between chains either because they preferentially react with other bis-lipids in the same chain or because of other competitive reactions. Sisson et al. reported evidence that is consistent with macrocyclization of bis-SorbPC lipids.[15] Intramolecular macrocyclization of the *sn*-1 and *sn*-2 reactive groups in the same lipid connects both lipid tails in a linear polymer (Figure 4b). The constrained nature of hydrated lipid assemblies reduces the number of conformations of lipid tails in a manner that increases the probability of macrocyclization in direct competition with cross-linking.

The cross-linking of (*E*,*E*)-DenPC lipids is significantly more efficient than the SorbPC lipids. The location of the dienoyl groups adjacent to the glycerol backbone was expected to be more favorable than SorbPC for cross-linking because the probability of macrocyclization is diminished when the reactive groups are in a more ordered region of the lipid molecule. When approximately one in eight (*E*,*E*)-DenPC lipids was a bis-substituted lipid, then cross-linking was observed. Since the degree of polymerization of polymer chains from mono-DenPC would be less than 50 for the polymerization conditions used in this study, and the minimum number of bis-lipids per polymer chain necessary for cross-linking is two, then the minimum mole fraction of bis-lipids required to observe cross-linking is >0.04. This suggests that under the experimental conditions employed here about a third of the bis-(*E*,*E*)-DenPC lipids react in a manner to cross-link poly(DenPC) chains.

Dienoyl lipids have been successfully used in the polymerization of bicontinuous cubic (Q_{II}) phases of hydrated lipids. Lee at al. employed a 3:1 molar mixture of a mono-DenPE and a bis-DenPC to form a Q_{II} phase at temperatures greater than 55 °C and then polymerized the Q_{II} phase in a manner that increased its thermal and chemical stability.[19] The poly(lipid) structure was not disrupted by organic solvents. Further support for the original suggestion that the poly-Q_{II} phase was cross-linked is provided by the current observations that indicate about one in eight of the dienoyl lipids must be bis-substituted to achieve cross-linking. Recently, Srisiri et al. described the formation and polymerization of a Q_{II} phase with *Ia*3*d* symmetry from a 9:1 molar mixture of a mono-Den monoacylglycerol and a bis-Den diacylglycerol.[26] Again the polymerization proceeded with retention of the Q_{II} phase; however, the poly(lipid) structure was soluble in selected organic solvents, indicating a lack of cross-linking of the Q_{II} phase. These data are consistent with the current study, which found that one bis-Den lipid in 10 Den-lipids is insufficient to achieve cross-linking.

The development of methods to polymerize hydrated lipid mesophases, i.e., lamellar, bicontinuous cubic, hexagonal, etc., can now be used to create new polymeric materials with novel properties.[1] Cross-linking polymerizations can substantially enhance the chemical and thermal stability of these materials. The capability of varying the cross-link density in these poly(lipid) structures by the use of mixtures of mono- and bis-substituted amphiphiles provides researchers with a means to further modify the physical properties of these polymers. Moreover, the ability to use high molar fractions of the monosubstituted lipids and still obtain cross-linked polymeric phases makes it straightforward to introduce various amounts of additional monosubstituted lipids that can subsequently be elaborated to create surface sites for the binding of molecules or ions for various biological or materials objectives.

Experimental Section

Methods and Materials. Compounds containing UV-sensitive groups were handled under yellow lights. ^{1}H NMR spectra were acquired on a Bruker AM-250 magnetic resonance spectrometer in chloroform-*d* or methylene chloride-d_2. UV−vis absorption spectra were recorded on a Varian DMS 200 spectrophotometer. Quasi-elastic light scattering (QELS) was performed with a BI 8000 autocorrelator from Brookhaven Instrument Corp., and particle sizes were calculated with the software accompanying the instrument.

1-Tetradecanol, pyridinium dichromate (PDC), dicyclohexylcarbodiimide (DCC), sodium hydride (60% dispersion in mineral oil), 4-(dimethylamino)pyridine (DMAP), potassium persulfate, sodium bisulfite, and Triton X-100 (TX-100) were obtained from Aldrich Chemicals. Trimethyl 4-phosphonocrotonate was purchased from Lancaster Synthesis Inc. 1-Palmitoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine and l-α-glycerophosphorylcholine:cadmium chloride (1:1) adduct were from Avanti Polar Lipids Co. DMAP was recrystallized from $CHCl_3$/ether (1:1), and the others were used without further purification. THF was distilled from sodium benzophenone ketyl. $CHCl_3$ and CH_2Cl_2 were distilled from CaH_2 under argon. The reactions were monitored by TLC visualized by a UV lamp or phosphomolybdic acid dye. The lipids were hydrated in Milli-Q water, Millipore Inc.

Synthesis. *Tetradecanal* (**1**). Pyridinum dichromate (PDC, 13 g, 34 mmol) was added to a solution of 1-tetradecanol (5 g, 22.6 mmol) in 500 mL of CH_2Cl_2. The reaction was stirred at room temperature overnight, and the mixture was filtered through silica gel to remove the PDC. The filtrate was concentrated, and the crude product was purified by column chromatography with hexane/EtOAc (95/5). A yield of 3.6 g (75%) was obtained. ^{1}H NMR ($CDCl_3$): 9.74−9.73 (t, $J = 1.83$ Hz, 1H), 2.42−2.36 (dt, $J = 7.35$, 1.83 Hz, 2H), 1.63−1.54 (m, 2H), 1.27−1.16 (b, 20H), 0.87−0.82 (t, $J = 6.56$ Hz, 3H) ppm.

Methyl 2,4-Octadecadienoate (**2**). Trimethyl 4-phosphonocrotonate (4.2 g, 20 mmol) in 50 mL of THF was added slowly to a suspension of NaH (60% dispersion in mineral oil, 1.4 g, 34 mmol) in 150 mL of THF at 0 °C under argon. The suspension was stirred until no evolution of gas was observed. Then, tetradecanal **1** (3.6 g, 17 mmol) in 100 mL of THF was added dropwise at 0 °C. The reaction was then allowed to warm to room temperature and monitored by TLC with hexane/EtOAc (98/2) as the mobile phase. After the reaction was completed, excess NaH was quenched by slow addition of cold water to the reaction. The THF was evaporated, and the residue was diluted with diethyl ether and then extracted

several times with water and brine. The organic layer was dried with anhydrous $MgSO_4$ and then concentrated. The crude ester was purified by column chromatography using hexane/EtOAc (98/2), affording 2.4 g of compound **2** (48% yield).

The ratio of (*E*,*E*)-2,4-dienoyl ester to its (*E*,*Z*)-isomer was determined by ^{1}H NMR to be 1/1. ^{1}H NMR ($CDCl_3$): 7.67−7.56 (dd, J = 15.22, 11.66 Hz, (*E*,*Z*)-isomer, 0.5H) and 7.32−7.27 (m, (*E*,*E*)-isomer, 0.5H), 6.17−5.76 (m, 3H), 3.75 (s, (*E*,*Z*)-isomer, 1.4H) and 3.74 (s, (*E*,*E*)-isomer, 1.6H), 2.31−2.25 (m, (*E*,*Z*)-isomer, 1H), 2.20−2.12 (m, (*E*,*E*)-isomer, 1H), 1.42−1.20 (b, 22H), and 0.9−0.85 (t, J = 6.57 Hz, 3H) ppm.

Methyl (E,E)-2,4-Octadecadienoate (**3**). A well-stirred solution of urea (4.4 g, 73 mmol) in 220 mL of methanol was treated with ester **2** (2.4 g, 4.1 mmol). The solution was kept at 0 °C overnight. The needlelike crystals were filtered, washed with cold methanol, and then dried under vacuum. These crystals were dissolved in ether and washed several times with water. The organic layer was combined and dried with anhydrous $MgSO_4$. After evaporation of ether, 1.2 g of the purified *E*,*E*-isomer **3** was obtained. ^{1}H NMR ($CDCl_3$): 7.32−7.22 (m, 1H), 6.17−6.12 (m, 2H), 5.82−5.76 (d, J = 15.37 HZ, 1H), 3.74 (s, 3H), 2.20−2.12 (m, 2H), 1.42−1.20 (b, 22H), 0.90−0.85 (t, J = 6.56 Hz, 3H) ppm.

2,4-(E,E)-Octadecadienoic Acid (**4**). A methanol solution of methyl ester **3** (1.2 g, 4.1 mmol in 50 mL of MeOH) was treated with 1.5 mol equiv of 85% aqueous solution of KOH. The mixture was refluxed overnight until the reaction was complete as determined by TLC with hexane/EtOAc (95/5). The MeOH solution was evaporated and ether was added. After the solution was acidified to pH 3 with dilute HCl solution, it was extracted several times with water. The organic layer was dried with anhydrous $MgSO_4$ and then concentrated. The crude acid was purified by recrystallization from hexane at −30 °C, giving 0.66 g of acid **4** in 57% yield. ^{1}H NMR (CD_2Cl_2): 7.40−7.30 (m, 1H), 6.24−6.22 (m, 2H), 5.81−5.75 (d, J = 15.26 Hz, 1H), 2.22−2.15 (m, 2H), 1.43−1.20 (b, 22H), 0.90−0.85 (t, J = 6.45 Hz, 3H) ppm.

1,2-Bis[2,4-(E,E)-octadecadienoyl]-sn-glycero-3-phosphocholine. l-α-Glycerophosphorylcholine:cadmium chloride (1:1) adduct (94.3 mg, 0.21 mmol), carboxylic acid **4** (100 mg, 0.36 mmol), and DMAP (43.6 mg, 0.36 mmol) were dissolved in 5 mL of CH_2Cl_2. Then, DCC (73.6 mg, 0.36 mmol) in 5 mL of CH_2Cl_2 was added. The mixture was stirred at room temperature in the dark for 3 days under argon. The white suspension was filtered to remove urea. Then, 3 g of Bio-Rad AG 501-X8 ion-exchange resin was added to the filtrate and stirred for 10 min. The resin was removed by vacuum filtration, and the filtrate was concentrated. The crude product was purified by column chromatography using hexane/EtOAc (9/1) followed by $CHCl_3$/MeOH/H_2O (65/25/2), giving 28 mg of lipid. ^{1}H NMR (CD_2Cl_2): 7.33−7.21 (m, 2H), 6.27−6.14 (m, 4H), 5.83−5.76 (dd, J = 12.51, 6.40 Hz, 2H), 5.29 (b, 1H), 4.46−4.23 (m, 4H), 4.02−3.98 (m, 2H), 3.71 (b, 2H), 3.32 (bs, 9H), 2.17 (b, 4H), 1.45−1.38 (b, 4H), 1.28 (bs, 40H), 0.91−0.87 (t, J = 6.54 Hz, 6H) ppm. MS: Calcd MW for $C_{44}H_{80}O_8NP$: 782.1. Found: *m*/*z* 782.6.

1-Palmitoyl-2-(2,4-(E,E)-octadecadienoyl)-sn-glycero-3-phosphocholine. 1-Palmitoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine (265.3 mg, 0.54 mmol), carboxylic acid **4** (100 mg, 0.36 mmol), and DMAP (43.6 mg, 0.36 mmol) were added in a flask with 5 mL of CH_2Cl_2. Then, DCC (73.6 mg, 0.36 mmol) in 5 mL of CH_2Cl_2 was added to the suspension. The reaction mixture was kept in the dark for 3 days and then worked up with the same steps as above to give 44 mg of product. ^{1}H NMR (CD_2Cl_2): 7.32−7.20 (m, 1H), 6.27−6.18 (m, 2H), 5.82−5.75 (dd, J = 15.01, 6.25 Hz, 1H), 5.24 (b, 1H), 4.44−4.17 (m, 4H), 3.95 (b, 2H), 3.76 (b, 2H), 3.34 (s, 9H), 2.34−2.26 (m, 4H), 1.61−1.54 (m, 2H), 1.45−1.38 (b, 2H), 1.27 (bs, 44H), 0.91−0.87 (t, J = 6.54 Hz, 6H) ppm. MS: Calcd MW for $C_{42}H_{80}O_8NP$: 758.1. Found: *m*/*z* 758.6.

Vesicle Polymerization. *Redox Initiation.* Large unilamellar vesicles (LUV) of polymerizable lipid were prepared as follow: A total of 0.5 mL of lipids from stock solutions (1 mM in water) was mixed and freeze-dried under high vacuum for 4 h. The dried lipid was then hydrated with deoxygenated Milli-Q water to a final concentration of 200 μM. Samples were vortexed to uniformity and subjected to five freeze−thaw−vortex cycles (−77 → 30 °C). The lipid suspension was extruded 5 times (2 × 0.2 μm−3 × 0.1 μm) through two stacked Nuclepore polycarbonate filters at 35 °C using a stainless steel extruder from Lipex Biomembranes.

The redox initiator was prepared from $K_2S_2O_8$ (27.0 mg, 0.1 mmol) and $NaHSO_3$ (10.4 mg, 0.1 mmol), which were weighed into a 10 mL volumetric flask and diluted. An aliquot (10 μL) was added to the LUV suspension, giving a [M]/[I] ratio of 5. The sample was sealed in an ampule with a septum and flushed with argon for 0.5 h. Polymerization was performed at 60 ± 2 °C in a water-circulating bath under a positive argon pressure for 18 h. Polymerization was monitored by UV absorption spectroscopy of aliquots diluted with Milli-Q water to ca. 60 μM.

Photopolymerization. The LUV, prepared as above, were placed into a Pyrex tube, thermostated at 40 °C, and 18.5 cm from a high-pressure Hg/Xe light source. Polymerization was monitored by UV absorption spectroscopy as above.

Surfactant Dissolution of Vesicles. The LUV were prepared as described above. After polymerization, the LUV were characterized by QELS for a 2 mL sample with a lipid concentration of 150 μM. An aliquot of 42.86 mM TX-100 solution was added. Each aliquot was equal to 2 equiv of lipid. The light-scattering intensities were determined again by QELS. TX-100 was added in 2 equiv increments up to a total of 12 equiv. Measurements at each concentration of TX-100 were performed at least three times. Light scattered (in photons/second) varied for each sample and was normalized as described by the following expression:

$$\text{normalized light scattering intensity} = (I - I_\infty)/(I_0 - I_\infty)$$

where I is the intensity of photons scattered after the addition of each equivalent of TX-100, I_∞ the intensity of photons scattered by a micellar suspension of TX-100 at a similar concentration, and I_0 the intensity of photons scattered by vesicles in the absence of TX-100. The average mean diameter of vesicles/particles was calculated by a nonnegatively constrained least-squares mathematical procedure.

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