Two-Dimensional Polymerization of Lipid Bilayers: Visible-Light-Sensitized Photoinitiation

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Received June 28, 1996; Revised Manuscript Received October 24, 1996⊗

ABSTRACT: The sensitized polymerization of synthetic lipid bilayers via visible-light irradiation of membrane-bound cyanine dyes is reported. Visible-light-initiated polymerizations were successful with liposomes composed solely or partly of either 1-palmitoyl-2-[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphatidylcholine (mono-SorbPC), 1,2-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphatidylcholine (bis-SorbPC), or 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphatidylcholine (DenPC). The ballasted sensitizing dyes reported here were conveniently incorporated into liposomes. The sensitizing dye *N*,*N*′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, DiIC(18)3, was used in many of these studies because its absorption properties were especially well matched to the spectral output of the light source. The lipid product from the sensitized photoconversion of mono-SorbPC was transesterified and analyzed by size exclusion chromatography, which revealed the presence of a polymer with a relative number-average degree of polymerization of 10^2. This value is similar to those reported for the AIBN-initiated polymerization of mono-SorbPC liposomes. The sensitized polymerization process is dependent on the presence of oxygen, and spin-trapping experiments demonstrated the formation of superoxide anion. The indivdual effects of pH, light intensity, and temperature suggest that the polymerization process is initiated by electron transfer from the dye excited state to oxygen, to yield superoxide anion, which in aqueous media combines to form hydrogen peroxide. In addition, it was shown that the monomeric lipids could be polymerized by addition of hydrogen peroxide at the experimental temperatures. These data suggest that the dye-sensitized polymerization is mediated by the formation of hydroxyl radicals in the immediate vicinity of the lipid bilayer. This polymerization chemistry appears likely to be compatible with oxygen-dependent systems such as living tissues.

Introduction

Lipids are amphiphilic molecules that self-organize into defined supramolecular structures when hydrated. Special features of these assemblies include a separation of the hydrophobic tails from water by aggregation of the chains and interaction of the lipid head group with water at the hydrophilic/hydrophobic interface. Alignment of the lipid tails creates a degree of order in the lipid systems that can be exploited to orient other molecules or to enhance reactions between the lipid molecules. The alignment of the reactive lipid groups creates a high local concentration as well as a nonrandom distribution of functional groups that facilitates polymerization in supramolecular assemblies.[1−4] Polymerizable lipids synthesized to date have incorporated vinylic monomeric units electrostatically associated with or covalently attached to the head group, near the lipid backbone, in the lipid tail interior, or at the end of the hydrophobic tail(s). The various methods to initiate bilayer polymerizations include direct photolysis and radical production by heat, UV light, or redox reactions. Lipid monomers with a reactive group in only one lipid tail produce linear polymers. Bis-substituted lipids may lead to cross-linking if lipid conformations allow both chains to react.[5] Recent reviews highlight the characteristics of lipid bilayer polymerization and the two-dimensional polymers formed.[1,6,7]

Lamparski and O'Brien published a systematic study of the two-dimensional polymerization of bilayers of phospholipids with terminal sorbyl monomer units on the lipid acyl chain(s), i.e. 1-palmitoyl-2-[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphatidylcholine (mono-SorbPC$_{16,17}$) and 1,2-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphatidylcholine (bis-SorbPC$_{17,17}$).[8] First, the degree of polymerization was determined as a function of initiator concentration using thermally produced radicals from AIBN as the initiating species. The number-average relative degree of polymerization at high conversion ranged from 50 to 300 and was found to be inversely proportional to the first power of the initiator concentration. This dependence on initiator concentration was similar to that reported for the corresponding acryloyl-substituted lipids, i.e. 1-palmitoyl-2-[12-(2′-propenoyloxy)dodecanoyl]-*sn*-glycero-3-phosphatidylcholine (mono-AcrylPC$_{16,16}$) and 1,2-bis[12-(2′-propenoyloxy)dodecanoyl]-*sn*-glycero-3-phosphatidylcholine (bis-AcrylPC$_{16,16}$).[9−11] Second, the rate of polymerization for direct UV photolysis of mono-SorbPC$_{16,17}$ and bis-SorbPC$_{17,17}$ was observed as a function of light intensity. The initial rate of polymerization was approximately first order with respect to irradiation intensity. In contrast to the AIBN-initiated polymerization, the direct photopolymerization of mono-SorbPC produced oligomers.[8] Tsuchida et al. described the effects of thermal and photoinitiation on the polymerization of 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphatidylcholine (DenPC) bilayer membranes and found that the direct UV photolysis produced much shorter polymers than were obtained by radical chain polymerizations.[12]

The variables of lipid structure, assembly structure, and monomer to initiator ratio affect the size of the polymers formed and resulting properties of the polymerized lipid assembly. Potential uses for polymerized bilayer membranes include photoinduced liposome fusion and/or leakage[13−15] and paramagnetic polymerized liposomes for enhanced magnetic resonance imaging.[16] The cross-linking polymerization of lipids has been successfully used to laterally reorganize lipids and the

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⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1996.

S0024-9297(96)00935-7 CCC: $14.00

dyes electrostatically associated with the assembly to facilitate excited state interactions of these bilayer associated components.[17]

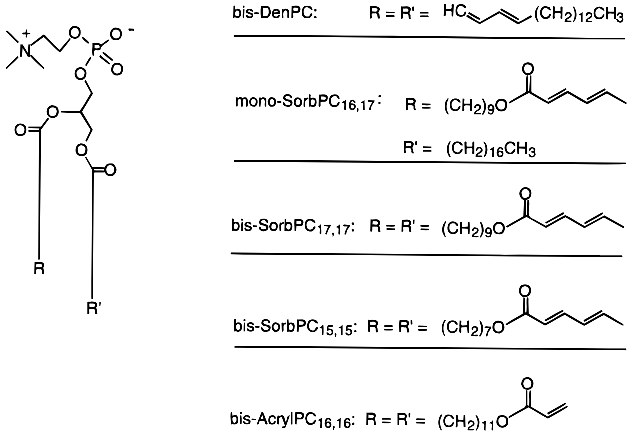
Dilution of a polymerizable lipid with a polymorphic, nonpolymerizable colipid has proven to be a useful strategy for the photopolymerization-induced leakage and fusion of liposomes.[13−15] Phosphatidylethanolamines (PE) form nonlamellar structures more readily than phosphatidylcholines (PC).[18] When an appropriate ratio of polymerizable PC to nonpolymerizable PE is employed, polymerization and subsequent reorganization of the bilayer lead to the formation of enriched domains of the polymorphic PE, thereby destabilizing the bilayer with release of the liposome encapsulated contents and/or the fusion of liposomes.[13,14] Other nonphotolytic methods of forming enriched domains of PE have been exploited for liposome destabilization.[19] The application of this phenomenon to controlled drug delivery is an active area of research. The use of radiation provides for temporal and spatial control of liposome destabilization. Clearly, the use of visible light for this potential phototherapy is more suitable than ultraviolet light because it avoids the use of high-energy irradiation. In addition, longer wavelength light penetrates tissue to a greater extent than shorter wavelength light.

The photosensitization of vinyl polymerization in solution, suspension, or thin films has proven important for several uses in photoimaging and biomedical applications. Extending the sensitivity of photoimaging systems to include visible light enhances their versatility and provides new options for irradiation sources. Recent reviews highlight various sensitizers and provide specific examples.[20−22] In some cases, excited state sensitizers react with activators, such as amines, to produce the initiating species.[23,24] If the sensitizers function via a triplet excited state,[25] then deoxygenated conditions are usually necessary.

The interest in phototherapeutics requires that the sensitization chemistry be compatible with oxygen; furthermore, it is preferable to have as few membrane-bound components as possible. A preliminary study of the cyanine dye−borate anion chemistry in lipid bilayers was undertaken. Cationic cyanine dyes in the presence of triaryl alkyl borate anions are especially effective visible-light-sensitizing photoinitiation systems with the unique property of long-wavelength sensitivity as well as good dark stability.[26−28] This two-component initiating system functions via electron transfer from borate anion to photoexcited cyanine dye. Upon donation of the electron, the oxidized borate radical quickly fragments to yield triarylborane and an alkyl radical initiator.[28] During the course of a series of control experiments, it became apparent that lipid bilayer membranes could be polymerized by irradiation of certain membrane-bound cyanine dyes in the absence of borate.

In this paper, we report the sensitized polymerization of synthetic lipid bilayers via irradiation of lipid assembly-bound cyanine dyes with visible light under ambient conditions. It is particularly notable that the polymerization occurs without addition of a borate activator and is dark stable. The primary objectives of this investigation include (1) the formation of polymerized supramolecular assemblies upon visible-light irradiation; (2) a study of the effects of controllable variables on the polymerization, i.e. temperature, pH, photolytic light intensity; and (3) determination of the probable mechanism of the sensitized initiation. The system employs amphiphilic cyanine dye(s) incorporated into lipid bilayers composed of purely polymerizable lipid or a mixture of polymerizable and nonpolymerizable lipid, such as those shown in Scheme 1. In addition, water-soluble cyanine dyes that are electrostatically bound to the membrane can be effective sensitizers. Visible-light photolysis in the presence of sensitizer and air leads to lipid polymerization as well as bleaching of the sensitizing dye. The wide range of absorption properties of the commercially available or synthetically accessible cyanine dyes permits tuning of the sensitization wavelength. This polymerization chemistry appears likely to be compatible with oxygen-dependent systems such as living tissues.

Scheme 1



Results

The sensitizing dyes reported here were conveniently incorporated into liposomes, and straightforward irradiation conditions were employed. The research employed SorbPC bilayers with sensitizing dye *N*,*N*′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, DiIC(18)3, for many of the studies because its absorption properties were especially well matched to the spectral output at 546 nm of the Hg(Xe) light source. Both DenPC and AcrylPC lipid bilayers as well as other sensitizers were examined and are discussed and compared to indicate the scope and limitations of this sensitization chemistry.

Interaction of Cyanine Dyes with Lipid Bilayers. The interaction of cyanine dyes with lipid bilayers was studied by absorption spectroscopy. The dye absorption spectra show a monomer band and a higher energy band that could be due to a vibrational band and/or due to the presence of H-aggregates. DiIC(18)3 in methanol is monomeric (Figure 1A). In contrast to the solution spectrum, the spectra of DiIC(18)3 in bis-SorbPC and DenPC bilayers show a somewhat more intense higher energy band that may be due to increased viscosity of the bilayer and/or due to dye aggregation. The extent of spectral change was dependent on the nature of the lipid and the method of dye incorporation into the bilayers. Cyanine dye was either added from a methanol stock solution to preformed liposomes (never exceeding 1% organic solvent by volume) or combined as a solid with the dry lipid prior to hydration. Equilibration of the dye molecules with *preformed* lipid bilayers was generally accomplished by sample incubation in the dark at 37 °C. During the incubation period the dyes equilibrate from an initial state of higher aggregation to a less aggregated state. The time required to reach an equilibrium state as

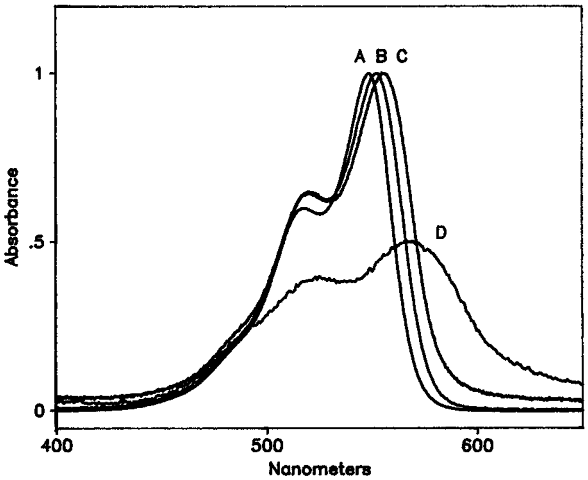


Figure 1. Absorption spectra of DiIC(18)3: (A) dissolved in methanol; (B) added to preformed bis-SorbPC$_{15,15}$ liposomes at a molar ratio of 10:1 lipid:dye; (C) combined and hydrated with bis-DenPC in a molar ratio of 7:1 lipid:dye; (D) added to preformed bis-DenPC liposomes at a molar ratio of 10:1 lipid: dye.

determined by the absorption spectrum was 5−10 min. After this time, the spectrum remained unchanged for at least 1.25 h. Incorporation of DiIC(18)3 into preformed SorbPC liposomes appeared to occur with little dye aggregation (Figure 1B). Addition of the same dye to preformed liposomes composed of DenPC often produced a large extent of dye aggregation (Figure 1D). The relationship between lipid structure and the tendency of ballasted cyanine dyes to aggregate in the lipid bilayer is not clearly understood. Since aggregated dyes were generally less effective sensitizers for the lipid conversions, we sought other ways to incorporate DiIC(18)3 into DenPC bilayers. Fortunately, the hydration of dried lipid and dye produced DenPC−dye bilayers that showed considerably less evidence of dye aggregation (compare Figure 1C to 1D).

Absorption properties of DiIC(18)3 were studied as a function of medium and extent of photoreaction of the monomeric lipid. The wavelength of maximum absorbance shifts as either variable is changed. The absorbance maximum of DiIC(18)3 shifts from 549 nm in methanol to 556 nm in bis-SorbPC bilayers. The sorbyl absorbance of the lipid shows a similar, but smaller shift when the dye is present (<3 nm). As the photosensitized reaction proceeds, a blue shift is observed in dye absorbance. This shift is on the order of 1.5−3 nm depending on the extent of bleaching and monomer loss. The changes in dye absorption spectrum shape also suggest the possibility of alterations in the extent of dye aggregation.

Visible-Light-Sensitized Photoreaction of Lipid Bilayers. Liposomes containing polymerizable lipid were prepared as described in the Experimental Section. Cyanine dye sensitizer was added to the liposomes, and the sample was irradiated with >470 nm light from the filtered output of a Hg(Xe) light source. Irradiation of undyed polymerizable lipid bilayers for up to 1 h produced no change in monomer absorbance, demonstrating that direct irradiation of monomer did not occur. Successful photosensitized reactions were also achieved using the monochromated output of a Spex fluorimeter Hg(Xe) lamp or the expanded 514 nm beam from an argon laser. All samples containing dye were handled in the dark before and after irradiation. The sample absorption spectrum varied with irradiation time (Figure 2), showing evidence of monomer and dye loss as well as photoproduct formation. The sorbyl monomer group has a λ_{max} near 258 nm, and the product of the dye-sensitized process absorbs maximally near 195 nm. The changes in monomer and product absorption during the reaction were similar to those reported previously for the AIBN-initiated polymerization of SorbPC bilayers.[8] The photosensitized reaction was inhibited when the sample was purged with argon gas to displace oxygen (Figure 3). The dye-sensitized photoreactions of SorbPC and DenPC liposomes were qualitatively similar; however, liposomes of bis-AcrylPC were unreactive under these conditions (Figure 3).

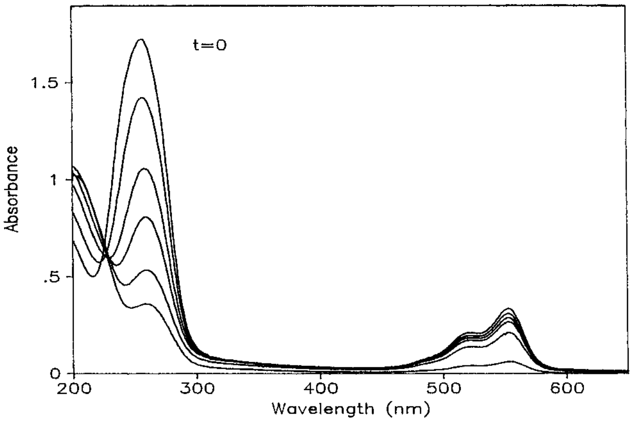


Figure 2. Absorption spectra of 20:1 bis-SorbPC$_{15,15}$:DiIC(18)3 liposomes at various exposures (4 min intervals) to visible light.

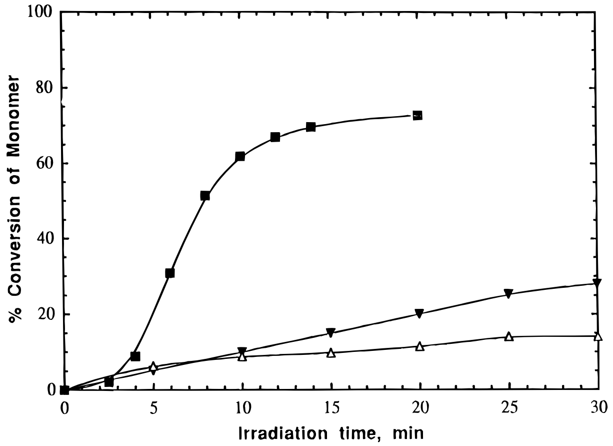


Figure 3. Percent conversion of monomer as a function of time for the DiIC(18)3-sensitized photoreaction of hydrated liposomes of bis-SorbPC$_{15,15}$ before (■) and after argon purging (▼) and bis-AcrylPC$_{16,16}$ (Δ) without argon purging.

The conversion of sorbyl monomer to product was estimated from the change in optical density of the monomer maximum near 258 nm. This absorption is due in part to the presence of cyanine dye and photoproduct chromophores that absorb in this same region. Figure 4 shows the UV/vis absorption spectrum of DiIC(18)3 before and after irradiation in unreactive saturated lipid bilayers (dimyristoylPC). Complete conversion of bis-SorbPC to oligomers (as judged by TLC) via direct photolysis causes 85% loss of absorbance at ca. 258 nm; thus the percent monomer loss at a given time (t) for sensitized photoreaction was estimated as follows:

$$\% \text{ monomer loss} = (A_0 - A_t)/0.85A_0$$

where A_0 and A_t are the sample optical densities at 258

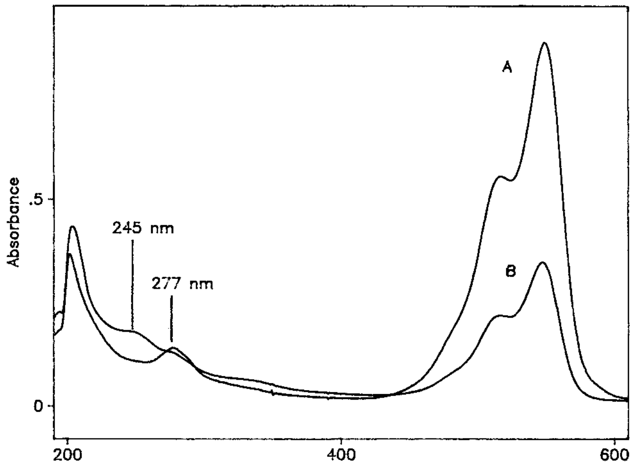


Figure 4. Absorption spectra of DMPC:DiIC(18)3 liposomes before (A) and after (B) 10 min of irradiation with visible light.

Table 1. Absorption Maxima and Extinction Coefficients for Bis-SorbPC$_{15,15}$ in Methanol and Lipid Bilayers (lipos)

	λ_{max} (nm)	ϵ (L mol^{-1} cm^{-1})
methanol	254	48 000
bis-SorbPC lipos	254	29 600
bis-SorbPC/DiI lipos	258−260	27 200
bis-SorbPC/DOPE lipos	254	30 800

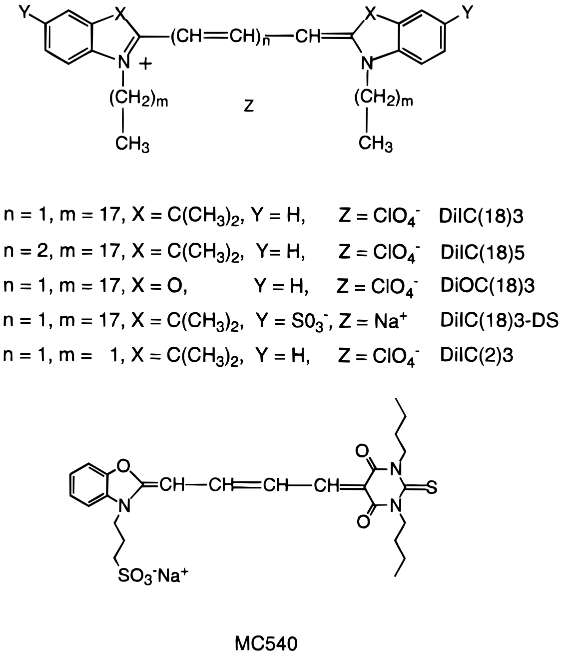
nm initially and at time t, respectively. However, during photosensitized reactions, the dye absorption (Figure 4) in the UV region prevents the absorbance at 258 nm from reaching the minimum value expected for complete loss of monomeric SorbPC. Therefore, the estimate of percent conversion calculated by the above expression is an *underestimate* of the actual value. Since the SorbPC extinction coefficient is sensitive to the sample environment,[29,30] it is neccessary to use the measured coefficients for lipid in methanol or bilayers (Table 1) to determine bis-SorbPC concentrations in the indicated media.

The rates at which the lipid and the dye are consumed depend on the ratio of the two components in the liposome. The half-life for bleaching of both components in liposomes of bis-SorbPC$_{15,15}$/DiIC18(3) decreases with decreasing dye content in the membranes. The consumption of the dye is more sensitive to the lipid/dye ratio than is lipid consumption.

Choice of Sensitizer. Dye-sensitized visible-light photoconversion of pure bis-SorbPC liposomes was successfully accomplished with each of the six amphiphilic and one water-soluble cyanine dyes in Scheme 2. Membrane association of the dye appears to be required for the reaction. The reaction was inefficient when the water-soluble N,N'-diethyl-3,3,3′,3′-tetramethylindocarbocyanine, DiIC(2)3, was used as the sensitizer with neutral SorbPC liposomes. However, if the liposomes were composed in part of an anionic lipid, then the electrostatically associated cationic DiIC(2)3 effectively sensitized the loss of monomeric SorbPC.

A comparison of sensitization by the green sensitizer, DiIC(18)3, and red sensitizer N,N'-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate, DiIC(18)5 (λ_{max} 649 nm), in liposomes composed of bis-SorbPC$_{15,15}$ (mole ratio of 14:1) showed that the DiIC(18)3-sensitized lipid conversion was faster ($t_{1/2}$ of 14 and 23 min, respectively) under the conditions employed, but the data are not corrected for the spectral output of the light source, which has a more intense output in the green than in the red. The sensitized polymerization of bis-SorbPC$_{15,15}$ liposomes with the blue-green sensitizer N,N'-dioctadecyloxacarbocyanine p-toluenesulfonate, DiOC(18)3 (λ_{max} 490 nm), was somewhat slower than that found with DiIC(18)3. Merocyanine 540 (MC540) was also found to sensitize the loss of bis-SorbPC$_{15,15}$ but to a lower extent than observed with DiIC(18)3.

Scheme 2



n = 1, m = 17, X = C(CH$_3$)$_2$, Y = H, Z = ClO$_4^-$ DiIC(18)3
n = 2, m = 17, X = C(CH$_3$)$_2$, Y = H, Z = ClO$_4^-$ DiIC(18)5
n = 1, m = 17, X = O, Y = H, Z = ClO$_4^-$ DiOC(18)3
n = 1, m = 17, X = C(CH$_3$)$_2$, Y = SO$_3^-$, Z = Na$^+$ DiIC(18)3-DS
n = 1, m = 1, X = C(CH$_3$)$_2$, Y = H, Z = ClO$_4^-$ DiIC(2)3

MC540

Photoproduct Characterization. The lipid product from the DiIC(18)3-DS-sensitized photoconversion of mono-SorbPC$_{16,17}$ was transesterified prior to further analysis by ^{1}H-NMR and size exclusion chromatography (SEC). Lyophilization of partially reacted mono-SorbPC$_{16,17}$ bilayers gave a film that was only slightly soluble in dichloromethane and mostly insoluble in benzene, in a manner that was similar to that observed for products from AIBN-initiated polymerizations of mono-SorbPC$_{16,17}$ liposomes. The molecular weight determination of zwitterionic polymers via SEC is difficult; therefore it is useful to cleave the polymer chains from the lipid head group and glycerol backbone. The photoproduct from mono-SorbPC$_{16,17}$ was benzene soluble after the transesterification reaction. By comparison with the ^{1}H-NMR procedure of Lamparski and O'Brien,[8] the average repeat unit molecular weight for the transesterified polymer was estimated to be 197 g/mol.

The SEC analysis of products from the transesterified photoproduct showed low molecular weight peaks, ascribed to the small-molecule esters, and a single high molecular weight peak that had a number-average molecular weight of 20 500 (relative to polystyrene standards). The high molecular weight peak had a polydispersity of 1.58. Based on the estimated repeat unit molecular weight, these values yield a relative number-average degree of polymerization of ca. 100. This value is similar to those reported for the AIBN-initiated polymerization of mono-SorbPC$_{16,17}$ liposomes, which ranged from 50 to 280 depending on the ratio of monomer to initiator in the bilayer.[8]

The poly(mono-SorbPC$_{16,17}$) from dye-sensitized polymerization was also characterized via TLC and ^{1}H-NMR. After irradiation, the suspension was poured into excess methanol, which was then evaporated. The resulting thin, red film was partly soluble in chloroform and methanol although some material was insoluble.

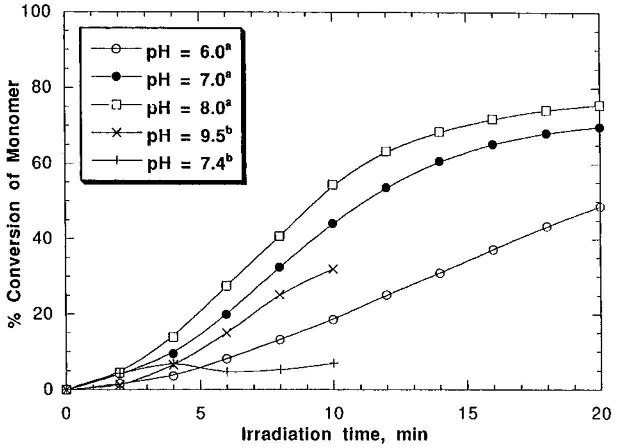


Figure 5. Percent conversion of monomer versus time as a function of pH for DiIC(18)3-sensitized polymerization of bis-SorbPC in (a) 20:1 bis-SorbPC$_{15,15}$:DiIC(18)3 and (b) 3:1:0.2 DOPE:bis-SorbPC$_{17,17}$:DiIC(18)3 liposomes.

Table 2. Rates of Sensitized Polymerization (R_p) and Dye Bleaching (R_b) in Bis-SorbPC$_{15,15}$:DiIC(18)3 Liposomes

lipid:dye	temp (°C)	[liposome] (nM)	$R_p \times 10^8$ (M s^{-1})	$R_b \times 10^9$ (M s^{-1})
21:1	15	~0.2	1.6	0.9
21:1	25	~0.2	2.1	1.3
23:1	35	~0.2	1.9	1.3
26:1	45	~0.2	1.6	1.7

TLC of the soluble fraction showed spots for unreacted dye and lipid as well as a streak extending from the unreacted lipid down to an immobile spot at the origin. ^{1}H-NMR of the soluble material indicates loss of sorbyl vinyl proton adjacent to the carbonyl (doublet at δ 5.72–5.80) and loss of the characteristic absorbance of the terminal methyl protons adjacent to the sorbyl unit (doublet at δ 1.85) in the lipid tail. The resonances match those observed for a sample of mono-SorbPC$_{16,17}$ which was polymerized by the thermal initiator AIBN,[8] the lone exception being the broad resonance at δ 2.4–2.7, which is likely due to the octadecyl tails of the cyanine dye.

Effect of pH on Rate of Polymerization. The DiIC(18)3-sensitized polymerization of pure bis-SorbPC$_{15,15}$ liposomes was studied as a function of pH over the range 6.0–8.0. The absorption spectrum of DiIC(18)3 was insensitive to this variation in pH. The observed rate of polymerization was greatest at the highest pH, the largest change occurring between pH 6 and 7 (Figure 5 and Table 2). The rates are 3.7 × 10^{-8}, 5.8 × 10^{-8}, and 7.0 × 10^{-8} M s^{-1} for pHs of 6.0, 7.0, and 8.0, respectively. A similar effect was observed with PC/PE liposomes at pH 7.0 and 9.5. The initial rates of the sensitized polymerization of bis-SorbPC$_{17,17}$/DOPE/DiIC(18)3 liposomes (Figure 5) were faster at pH 9.5 than at 7.0 (5.8 × 10^{-8} and 1.1 × 10^{-8} M s^{-1}). Thus the pH effect is independent of the head groups of the PC and PE lipids. Direct UV photolysis of bis-SorbPC-containing bilayers does not display a pH dependence (unpublished data).

Effect of Liposome Concentration on the Rates of Polymerization and Sensitizer Bleaching. The sensitized polymerization of bis-SorbPC$_{15,15}$ by DiIC(18)3 was performed at various liposome concentrations while maintaining a constant lipid to dye ratio. Liposome concentrations were determined from the liposome size derived from dynamic laser light scattering and calculating the relationship between lipid concentration and liposome concentration.[31] The percent loss of monomer per time of exposure was virtually unaffected by the liposome concentration in the range 0.15–0.92 nM. However, percent bleaching of the sensitizer was affected by liposome concentration in an inverse manner. These data suggest that the processes responsible for dye bleaching is sensitive to liposome–liposome interactions.

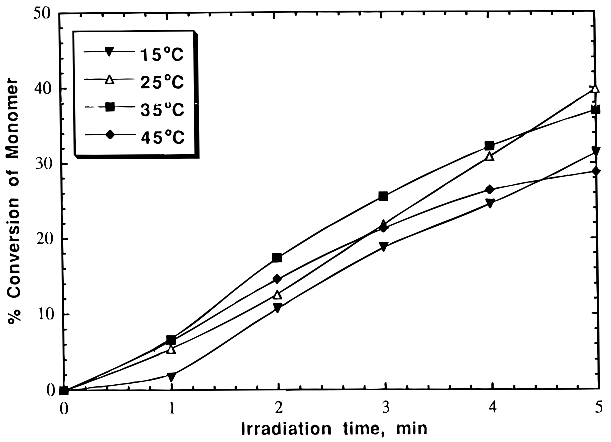


Figure 6. Percent conversion of monomer versus time as a function of temperature for DiIC(18)3-sensitized photoreaction of hydrated liposomes of bis-SorbPC$_{15,15}$ (23:1 lipid:dye).

Effect of Temperature on Sensitized Polymerization. The rate of DiIC(18)3-sensitized polymerization of bis-SorbPC$_{15,15}$ bilayers showed only a 25% change over the temperature range 15–45 °C (Figure 6 and Table 2), which spans the T_m of the lipid, 18.5 °C.[32] The lateral diffusion coefficient of lipid bilayer components is known to increase by 2 orders of magnitude as the sample temperature is increased from below to above the lipid T_m. Therefore the sensitized polymerization process is not sensitive to the lateral diffusion of bilayer-bound reactants. The rate of dye bleaching increased 40% as the temperature was increased from 15 to 25 °C and doubled between 15 and 45 °C (Table 2).

Effect of Incident Light Intensity on the Rate of Polymerization. Liposomes composed of either bis-SorbPC$_{15,15}$/DiIC(18)3 or bis-SorbPC$_{17,17}$/DOPE/DiIC(18)3 were irradiated at a variety of light intensities, and the rates of polymerization were determined from plots of bis-SorbPC concentration versus time (Figure 3), ignoring the slow initial rate that is a function of the initiation process. The final slow stage of the polymerization may be due to an increase in viscosity (decrease in rate of lateral diffusion in the bilayer) and decreased monomer concentration. The rate of polymerization was linearly related (least-squares fit with a correlation coefficient of 0.997) to the incident light intensity (Table 3 and Figure 7).

Effect of Hydrogen Peroxide on SorbPC Liposomes. Bis-SorbPC$_{15,15}$ liposomes, [lipid] = 21.7 μM, in thermostated water were combined with hydrogen peroxide, 55 mM, and the sample absorption spectrum was measured at various times. The characteristic sorbyl chromophore progressively decreased in intensity. The loss of monomeric lipid was faster at higher temperatures. Approximately half the lipid was transformed in 40 min at 37 °C. This dark reaction was inhibited by the addition of D-mannitol, a known hydroxyl radical scavenger.

Spin Trapping of Radical Species. In order to probe the possible intermediate species in the photo-

Table 3. Rates of Sensitized Sorbyl Polymerizations in (a) 20:1 Bis-SorbPC$_{15,15}$:DiIC(18)3 and (b) 3:1:0.1 DOPE:Bis-SorbPC$_{17,17}$:DiIC(18)3 Liposomes as a Function of Incident Light Intensity from the Filtered Output of a 200 W Hg(Xe) Lamp and (c) 13:1 Bis-SorbPC$_{15,15}$:DiIC(18)3 Liposomes Irradiated with 545 nm Light from a 450 W Hg(Xe) Lamp (14.4 nm Band-pass Monochromator)

I_{int} (photons/s $\times 10^{-18}$)	% rel transmittance	R_p (mol L^{-1} s^{-1} $\times 10^{8}$)
1.28[a]	100	10
1.18[a]	92.2	8.9
0.67[a]	52.3	4.9
0.97[b]	75.8	5.3
0.48[b]	37.5	4.6
0.24[b]	18.8	2.6
0.036[c]	2.8	3.5

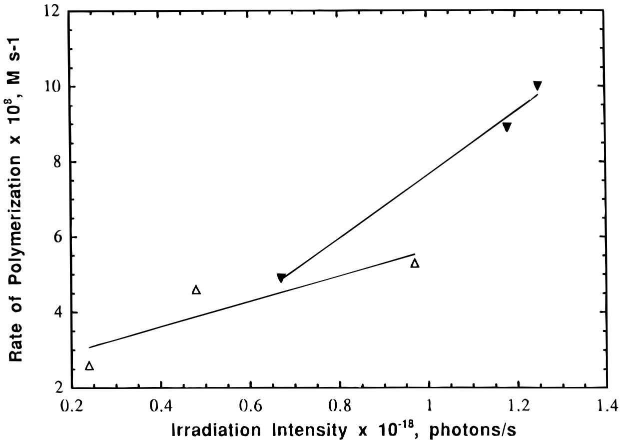


Figure 7. Rate of polymerization as a function of light intensity for DiIC(18)3-sensitized polymerization of bis-SorbPC liposomes with (△) and without (▼) DOPE diluent.

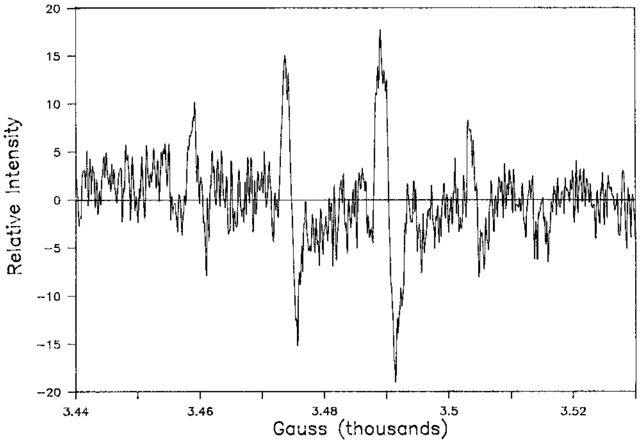


Figure 8. EPR spectrum obtained after 40 min of irradiation of DiIC(18)3 in DMPC liposomes in the presence of 10 mM DMPO and 1 M DMSO; [DiIC(18)3] = 50 μM.

sensitization mechanism, EPR analysis was employed to detect radical species. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was added to a suspension of DMPC/DiIC(18)3 liposomes as described in the Experimental Section. Following irradiation of the sample with visible light, it was transferred to an EPR spectrometer for analysis. A signal corresponding to the hydroxyl spin adduct of DMPO was observed. This spectrum could be a consequence of trapping either OH$^\bullet$ or O$_2^{\bullet-}$ since the O$_2^{\bullet-}$/DMPO spin adduct quickly degrades to the OH$^\bullet$/DMPO derivative.[33] To ascertain which of these two possible intermediates (OH$^\bullet$ and O$_2^{\bullet-}$) was initially formed, the experiment was repeated with 1 M DMSO included in the sample. Hydroxyl radicals quickly react with DMSO to generate a methyl radical, which reacts with DMPO to produce a substantially different EPR signal pattern than observed for the hydroxyl spin adduct. Irradiations of DMPC/DiIC(18)3 bilayers in the presence of DMPO and DMSO gave the same signal as in the absence of DMSO (Figure 8), indicating that the initially generated species from the visible light photolysis of DiIC(18)3 in lipid assemblies is the superoxide anion.

Quantum Efficiencies of Monomer and Dye Consumption. Using the method described in the Experimental Section, the efficiency of monomer and sensitizer consumption was determined as a function of the number of photons absorbed using chemical actinometry to determine the photon flux at the cuvette front and sample optical density to determine the percent of light absorbed. Bis-SorbPC$_{15,15}$:DiIC(18)3 (ca. 17:1) liposomes were irradiated with 545 nm monochromated light from a 450 W Hg(Xe) lamp through 8 mm monochromator entrance and exit slits with a band-pass of 14.4 nm. Quantum efficiencies (Φ) were determined from the calculated number of photons absorbed and the number of monomer and sensitizer molecules bleached/reacted as determined from changes in absorbance. The photon flux at the cuvette face was determined to be 3.3×10^{16} photons/s. The dye absorbance at 545 nm was 0.486 corresponding to 67.3% of the incident light absorbed by the sample, or 2.2×10^{16} photons/s absorbed. Bis-SorbPC$_{15,15}$ monomer was 6.5% converted to polymer during a 300 s irradiation (2.0×10^{13} monomers/s), yielding a quantum efficiency of lipid conversion (Φ_L) of 9.0×10^{-4} monomers reacted/photon absorbed. Similarly, 1.3% dye was bleached during the 300 s irradiation (6.0×10^{11} dye/s), producing a quantum efficiency of dye consumption (Φ_D) of 2.7×10^{-5} dyes bleached /photon absorbed. The ratio of Φ_L to Φ_D is a measure of the overall efficiency of the reaction in terms of the number of lipid molecules consumed per dye bleaching event. This corresponds to 33 lipid monomers reacted/dye molecule bleached.

Discussion

The polymerizations of bis-SorbPC or bis-DenPC bilayers were previously reported by direct irradiation or by radicals generated from thermal or redox initiators.[8,12,34] This report describes a method to sensitize these lipid polymerizations to visible light in the presence of oxygen. The experimental results indicate that photoactivation of membrane-bound cyanine dyes leads to bilayer polymerization of DenPC and SorbPC lipid monomers. The irreversible bleaching of the UV absorption band of the lipid provides clear evidence of disruption of the conjugation in the reactive groups. It has been shown that similar spectral results (i.e. decreased optical density at the maximum but increased optical density at shorter wavelengths) for direct irradiation of SorbPC lipids correspond to polymerization of the lipid tails. Moreover, the faster rate of reaction of the lipid compared to the dye in many of the experiments suggests that the monomer is being consumed by a chain process. Several cyanine dyes with absorption maxima in the blue, green, or red regions of the visible spectrum can sensitize the polymerization of lipid assemblies. This versatility provides the ability to vary the wavelength of the exciting light to meet various experimental requirements. Nearly complete conversion of monomer to polymer can be accomplished in pure polymerizable lipid bilayers in a few minutes.

All but one of the monomeric lipids studied were successfully polymerized using DiIC(18)3. Each of these reactive lipids have an activated diene group that is either incorporated in the lipid tail near the glycerol backbone (bis-DenPC) or at the end of the hydrocarbon tail (mono- and bis-SorbPC). Sorbyl monomers polymerize faster than DenPC monomers under the conditions investigated. This suggests either that the sorbyl functionality located at the end of the lipid tail is more reactive or that the initiating species is sufficiently hydrophobic that it preferentially partitions into the bilayer interior and is present at much lower concentrations at the lipid−water interface than in the bilayer interior. Inefficiencies due to the somewhat greater dye aggregation in DenPC bilayers would also tend to reduce the polymerization rate. In contrast to the SorbPCs and DenPC, the AcrylPC did not polymerize under the described sensitization conditions. Since the polymerization of acryloyl monomers is readily inhibited by oxygen, the cyanine dye−oxygen sensitization protocol is largely ineffective for acryloyl-substituted lipids. Thus the oxygen requirement of the cyanine dye sensitized polymerization limits the types of monomers that can be usefully employed.

In order to characterize the nature of the dye-sensitized reaction, the photoproducts were isolated and examined by TLC, NMR, and SEC. Each of the methods indicated the presence of high molecular weight products. The relative number-average degree of polymerization of the polymeric products was estimated by SEC to be ca. 10^2. Note that the liposome structure does not limit the size of these polymers since a 100 nm diameter liposome consists of approximately 8.0×10^4 lipid monomers. The observed degrees of polymerization for the photosensitized polymerization products are considerably greater than those for the oligomers obtained by direct UV-initiated polymerization of mono-Sorb-$PC_{16,17}$ and are comparable to those reported for the AIBN-initiated polymerizations of mono-$SorbPC_{16,17}$.[8] The NMR data also indicate that the dye-sensitized photoproducts were similar to those obtained by AIBN-initiated polymerizations of this same monomer. These data suggest that the initiating species is a radical.

The rates of sensitized polymerization and dye bleaching are affected by many different variables, including choice of sensitizer, temperature, pH, concentration of monomer in bilayer, and incident light intensity. The data in Table 2 indicate the polymerization rate is little affected by changes in temperature, either above or below the lipid T_m, thereby suggesting that changes in lipid lateral diffusion coefficient by 2 orders of magnitude do not significantly affect the initiation and propagation steps. The apparent low activation energy of the photosensitized polymerization implicates a photoreaction as the rate-determining step, rather than some thermal transformation of a photoactivated species. However, changes in irradiation intensity, light source, and concentration of monomeric lipids in the bilayer do modulate the polymerization rate (Tables 2 and 3). Dye bleaching is enhanced under aggregated conditions and higher liposome concentrations. However, as liposome concentration increases, the rate of polymerization increases much more rapidly than the dye bleaching rate. This corresponds to a more efficient sensitized polymerization in terms of dye consumption.

The lipid environment alters the properties of the amphiphilic cyanine dye absorption, emission, and aggregation. Absorption maxima are shifted to lower energy and fluorescence emission spectral shape is slightly altered. The aggregation state of the dye, in the concentration range studied, is enhanced by incorporation into the lipid assembly when compared to methanol solution. Previous studies on the photophysical properties of similar cyanines in lipid assemblies report changes in cyanine photophysics due to the increased viscosity and lower dielectric constant of the lipid environment as compared to certain organic solvents.[35,36] These reports suggest that the loss of mobility and increase in aggregation state caused by incorporation of the dyes into lipid assemblies produces inhibition of photoisomerization and enhancement of fluorescence. This is a similar effect to that observed when the cyanine dye is placed in a rigid environment by either chemical modification of the dye structure or increasing the viscosity by gelling the medium.[37,38]

A most unusual characteristic of the lipid−dye interaction studied here is the strong affinity of DiIC(18)3 for SorbPC bilayers. Not only does the dye incorporate into SorbPC assemblies in a much less aggregated manner than with other lipids, the dye apparently exchanges between liposomes with a preference for the SorbPC bilayers. This phenomenon will be reported elsewhere. This effect indicates there is a favorable interaction between the cyanine dye and SorbPC, which may be due to compatible chain conformations of the dye and lipid tails.

The observation that the photosensitization requires the presence of oxygen indicates the mechanism involves the interaction of excited dye and oxygen to form a reactive intermediate. Since changing the counterion from perchlorate to *p*-chlorobenzenesulfonate did not affect the reaction rate of DiIC(18)5-sensitized SorbPC polymerization, counterion participation was ruled out. The two most likely interactions between excited dye and oxygen are production of singlet oxygen through triplet energy transfer or production of superoxide anion via electron transfer. The possibility of energy transfer to molecular oxygen seems unlikely because the reported quantum yields for singlet oxygen formation in similar systems are reportedly very modest.[35] Furthermore, energy transfer would regenerate ground state dye, whereas in the experiment reported here, dye bleaching is observed. In our experiments, the EPR data indicate superoxide anions are formed during the irradiation of DiIC(18)3 in nonpolymerizable DMPC liposomes. The production of $O_2^{\bullet-}$ could occur via the electron transfer reaction in eq 1.

$$^{*}\mathrm{DiIC(18)3^{+}} + \mathrm{O_2} \rightarrow \mathrm{DiIC(18)3^{\bullet 2+}} + \mathrm{O_2^{\bullet -}} \quad (1)$$

The standard redox potential for oxygen/superoxide anion is −0.33 eV in water.[39] The excited states of the cyanine dyes studied here are all good enough electron donors to participate in eq 1.

The sensitized polymerization is inhibited at low pH, implicating the deprotonated form of superoxide anion, rather than the hydroperoxy radical, as a reactive species in the formation of an initiator. There are two pH-sensitive processes that diminish the concentration of superoxide anion: the protonation of superoxide anion (eq 2) and the self-dismutation reaction (eq 3). High pH shifts the equilibrium of both to the left, favoring a higher concentration of superoxide anion. Note that the pK_a of $HO_2^{\bullet}$ is 4.69.[39]

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet} \quad (2)$$

$$O_2^{\bullet -} + HO_2^{\bullet} \rightarrow O_2 + HO_2^- \quad (3)$$

The initiating species could be either superoxide anion or hydroxyl radical. The superoxide anion itself may serve as the initiator; however, no previous reports of superoxide anion as an initiator could be found, and attempts to generate $O_2^{\bullet -}$ directly by other means and thereby initiate bis-SorbPC polymerization failed. In addition, it is unclear whether superoxide anion permeates into and through phospholipid bilayers. Some reports indicate the permeability is low or absent.[40,41] In contrast, its permeation across dioctadecyldimethylammonium chloride bilayers was recently reported and found to occur more rapidly at temperatures above the lipid T_m.[42]

The photosensitized polymerization of SorbPC requires a phospholipid membrane permeant initiating species. Unpublished observations in our laboratory clearly show that hydroxyl radicals are membrane permeable. Even when generated by redox reactions in the aqeous media outside a lipid assembly, such as a multilamellar liposome (MLV), the polymerization of lipid throughout the MLV is observed. Perhaps the superoxide anion produced initially (EPR data) proceeds to generate hydroxyl radical. One possiblity is shown in eq 4, where two superoxide anions combine to form hydrogen peroxide. The equilibrium for this reaction is reported to be 4×10^{20} at pH 7.[38] Even at pH 9 the equilibrium favors the production of hydrogen peroxide, which would then spontaneously decay to give two hydroxyl radicals. Recall that polymerization of bis-SorbPC was observed when hydrogen peroxide alone was used as a hydroxyl radical source.

$$2O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (4)$$

Equation 4 predicts the formation of two hydroxyl radicals from two superoxide anions which are in turn the result of two excited cyanine molecules. This pathway appears to be consistent with the observed light intensity dependence.

Conclusions

This research sought to sensitize the polymerization of lipid assemblies to visible light. Furthermore, it was desirable to find a chemistry that was suitable for physiological conditions: 37 °C, pH = 7.4, and ambient oxygen conditions. The final requirement presented the greatest challenge since oxygen is an effective inhibitor of radical polymerizations. The chemistry described here requires oxygen to initiate the reaction. This clearly limits the reaction to selected monomers. In spite of these limitations it is still very effective for cross-linking polymerizations of appropriately designed bis-substituted lipids. We have already shown that cross-linking polymerizations of sorbyl and dienoyl lipids can be used to stabilize bilayer as well as nonbilayer lipid assemblies. Futhermore, under appropriate circumstances cross-linking polymerizations can be usefully employed to trigger the release of water-soluble agents from liposomes.[13−15] In due time we will report the effect of cyanine dye sensitized cross-linking reactions for both the stabilization and destabilization of lipid assemblies.

Experimental Section

Materials. The polymerizable lipids 1-palmitoyl-2-[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphatidylcholine (mono-Sorb-$PC_{16,17}$), 1,2-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphatidylcholine (bis-$SorbPC_{17,17}$) and 1,2-bis[8-(2′,4′-hexadienoyloxy)octanoyl]-*sn*-glycero-3-phosphatidylcholine (bis-$SorbPC_{15,15}$) were generously provided through the synthetic efforts of Henry Lamparski using the procedures described previously.[14] 1,2-Bis[12-(2′-propenoyloxy)dodecanoyl]-*sn*-glycero-3-phosphatidylcholine (bis-Acryl-$PC_{16,16}$) was synthesized by the procedures reported earlier.[10] 1,2-Dimyristoyl-*sn*-glycerophosphocholine (DMPC) was purchased as a 20 mg/mL solution in $CHCl_3$ from Avanti Polar Lipids, Inc., and stored at −40 °C. Lipid purity was confirmed by thin-layer chromatography (TLC) with chloroform/methanol/water (65:25:4 by volume). Pure lipids eluted to a single spot with an R_f of 0.35−0.40. Stock benzene solutions of polymerizable lipids (ca. 20 mg/mL) were stored at −40 °C as an amorphous ice.

Cyanine dyes were purchased from Molecular Probes. Concentrations in methanol were determined via visible absorption spectroscopy using literature values for extinction coefficients. Purity was confirmed by UV/vis spectroscopy as well as TLC on plates pretreated with 5% $NaClO_4$ in methanol and eluted with $CHCl_3$ containing 5% of the $NaClO_4$/methanol solution by volume. Dyes were stored at −40 °C as solids or in methanolic solution.

HPLC grade methanol and spectrophotometric grade methanol were purchased from J. T. Baker and used as received. Trisodium phosphate (98+%) and sodium chloride for buffer preparations were purchased from Aldrich and Mallinckrodt, respectively, and used as received. Hydrogen peroxide, 30% solution, reagent grade, was purchased from VWR, stored at 4 °C, and used as received.

5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was obtained from Aldrich and stored at −40 °C. Dimethyl sulfoxide (DMSO), 99.9% HPLC grade, was purchased from Sigma-Aldrich and used as received.

Rienecke's salt was purchased from Sigma with ammonium as the counterion and stored at room temperature. The potassium derivative was produced via ion exchange in a KNO_3 aqueous solution.[43] The product was recrystallized from cold Milli-Q water containing KNO_3 and stored as crystallized at −40 °C. KNO_3, perchloric acid ($HClO_4$), and $Fe(NO_3)_3$ were obtained from Curtin Matheson Scientific, Inc., Fisher Scientific Co. and J. T. Baker, respectively, and used as received.

Methods. Liposome Preparation. Large unilamellar liposomes and multilamellar liposomes were prepared as follows: approximately 3 mg of dried lipid film was obtained from a stock solution in benzene (10 mg/mL) by passing a gentle stream of argon over the sample followed by drying under vacuum for at least 4 h. If dye was to be incorporated in all leaflets of lipid bilayers, an aliquot of dye solution was added to the lipid solution to yield the appropriate ratio of lipid to dye and the drying procedure was repeated. All steps performed in the presence of dye were carried out in the dark. Phosphate buffer solution (PBS, 10 mM phosphate, 150 mM NaCl, pH 7.5) was added to hydrate the lipids to a concentration of approximately 1 mg/mL. Vortex agitation led to release of the lipid film from the flask walls and generation of a homogeneous suspension. Ten freeze/thaw cycles consisting of immersion of the sample in a −70 °C dry ice/2-propanol bath until frozen followed by thawing in a room temperature water bath (approximately 8 min) produced extended lipid bilayers. Extrusion of the lipid suspension through two stacked 0.1 μm or 0.6 μm Nuclepore filters ten times at 37 °C and 250 or 100 psi gave unilamellar or oligolamellar liposomes, respectively.[44] Extrusion was performed using a stainless steel extruder from Lipex Biomembranes. The lipid concentration of SorbPC was determined by adding an aliquot of liposome suspension to methanol and obtaining a UV absorption spectrum (ϵ_{254nm} = 48 000) on a Varian DMS 200 double-beam UV/visible spectrophotometer. The bilayers containing dye were dissolved in methanol and the absorption spectrum was used to determine the dye concentration. If the liposomes were prepared in the presence of dye, some dye was lost during extrusion due to dye adherence to the Nuclepore filters precluding control of the lipid:dye ratio.

The addition of dye to preformed liposomes was used to incorporate dye into the outer bilayer leaflet of the liposome. After formation of liposomes, an appropriately sized sample was prepared, typically 1 mL of 60 μM lipid to yield a useful optical density (<2.0). An aliquot of dye in methanol was added directly to the liposome suspension such that the volume of organic solvent was <1% of the total volume. The sample was incubated at the desired temperature for 15 min to allow equilibration of the dye with the lipid bilayer. The UV/vis spectra of the suspension indicated that the dye was partly aggregated at the outset and became progressively less aggregated until equilibrium was reached during the 15 min incubation.

Visible-Light Irradiation. Samples were unstirred and thermostated at 37 °C throughout experiments unless otherwise stated.

(1) Hg(Xe) Lamp. Samples were irradiated in cuvettes of 1 cm path length using the visible light produced by the filtered output of a 200 W Hg(Xe) arc lamp (Corning filter CS3−72, $\lambda < 470$ nm). Power was measured at the front of the sample cuvette with a Coherent 210 power meter. Light intensity was varied to determine the relationship between intensity and polymerization rate using optical density filters placed directly after the cut-off filter.

(2) Argon Laser. The 514 nm light of a 2 W Coherent argon Ion laser was directed onto the sample cuvette through a beam expander to increase the diameter from 1.5 to 19.1 mm and exposing the entire front of the cuvette.

(3) Monochromated Hg(Xe) Lamp. Light from a 450 W Hg(Xe) lamp was passed through a Spex double-grating monochromator with slit widths of 8 mm and a band-pass of 14 nm. Irradiation light intensity was determined using chemical actinometry as described in this section.

Polymerizations were monitored by UV/vis spectroscopy. The percent conversion of sorbyl monomer to polymer was estimated from the change in optical density of the monomer maximum as described in the Results section.

H_2O_2 Initiation. H_2O_2 was added to a bis-SorbPC$_{15,15}$ lipid suspension at 37 °C in a 2500:1 H_2O_2:bis-SorbPC ratio. UV absorption spectra were obtained every 10 min to monitor the polymerization. H_2O_2 was included in the reference cuvette to correct for its UV absorbance.

Transesterification of Polymerized Lipids. Following polymerization of the lipid bilayers, the water was removed by lyophiphilization to leave a dry, fine powder. This polymerized lipid sample was transesterified as previously described[8,30] to enhance the polymer solubility for SEC and ^{1}H-NMR analysis.

Characterization of Polymeric mono-SorbPC$_{16,17}$. The products of irradiation of mono-SorbPC$_{16,17}$ bilayers containing DiIC(18)3 were investigated by TLC and ^{1}H-NMR spectroscopy. The liposomes were prepared in Milli-Q water (pH adjusted to 7.1 by addition of NaOH). Concentrated samples of liposomes were irradiated for 1 h. The samples were then poured into excess methanol followed by evaporation of the solvent. A thin, red film was left behind which was mostly soluble in chloroform. The chloroform-soluble material was analyzed by TLC ($CHCl_3$:methanol:H_2O, 65:25:4 by volume as eluent) and ^{1}H-NMR spectroscopy.

Size Exclusion Chromatography. The relative molecular weight of the transesterified polymer was determined by SEC using a Waters Maxima 820 chromatograph with an Ultrastryragel 10^3 Å column having an effective molecular weight range of 2×10^2 to 3×10^4. Tetrahydrofuran (THF) was used as the mobile phase and was filtered through 0.45 μm Waters nylon filters and purged with helium. A Waters Model R401 differential refractometer served as detector, and the system was interfaced to a NEC Powermate 1 computer with Maxima 820 version 3.31 software. Calibration and analyses were performed as described by Sells and O'Brien.[10]

Electron Paramagnetic Resonance Spectroscopy. A 2 mM solution of DMPO in phosphate buffer solution was prepared. Pure DMPC and DMPC/DiIC(18)3 (dye in both leaflets) liposomes were prepared in PBS. Suspensions were then diluted with PBS and PBS/DMPO to a final concentration of 10 mM DMPO and 50 μM DiIC(18)3 (when applicable) and 1 M DMSO (when applicable). Irradiations were performed as before with the filtered output of a 200 W Hg(Xe) arc lamp at 37 °C. Following polymerization, an aliquot of the sample was transferred to a quartz EPR capillary for analysis. Spectra were obtained on a Bruker ESR300 with the following spectrometer settings: microwave power 2.00 mW, modulation frequency 100 kHz, modulation amplitude 2.851 G, sweep 35.8 G/min, and receiver gain 2.00×10^5.

Chemical Actinometry. The quantum efficiency of the polymerization was obtained via chemical actinometry using Reinecke's salt as described by de Mayo.[43] The lamp flux (photons/s) from the monochromated output of a 450 W Hg-(Xe) lamp was determined using the potassium version of Reinecke's salt. An aqueous solution of Reinecke's salt was prepared in Milli-Q such that the absorbance was greater than 2.2 optical density units. $Fe(NO_3)_3$ (5%) in 1.2 M $HClO_4$ was prepared by diluting 70% (12.3 M) $HClO_4$ to the appropriate concentration and adding enough solid ferric nitrate to yield a 5 mol % solution. Reinecke's salt produces thiocyanate ion upon irradiation. The ion can be detected spectrophotometrically as a complex with ferric ion. Quantum efficiencies, defined as the number of monomer or dye molecules consumed per photon absorbed, were calculated from the flux determined from chemical actinometry and the number of monomer and dye molecules consumed per second derived from UV/vis spectra.

Acknowledgment. The authors wish to thank Dr. Henry Lamparski for his generous contribution of synthetic polymerizable lipids and Dr. Arnold Raisimring for helpful discussion and assistance with EPR analysis. This research was supported by grants from NSF-DMR and NIH-GM 40427.

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MA960935T