Effect of Vesicle Size on the Polymerization of a Diacetylene Lipid

Brian M. Peek,† John H. Callahan,‡ Krishnan Namboodiri,† Alok Singh,† and Bruce Paul Gaber*,†

Center for Bio/Molecular Science and Engineering, Code 6900, and Chemistry Division, Code 6113, Naval Research Laboratory, Washington, D.C. 20375

Received March 1, 1993; Revised Manuscript Received September 30, 1993®

ABSTRACT: The effect of vesicle size on the photopolymerization products of the polymerizable diacetylene lipid 1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) has been examined using size exclusion chromatography (SEC) and mass spectrometry. Lipid polymers from 50-, 100-, 300-, and 600-nm vesicles have the same molecular weight range with a prominent polystyrene equivalent molecular weight peak between 1.6×10^6 and 2.2×10^4. The observed intensity of this peak increased as vesicle size was increased from 50- through 300-nm diameter, but was decreased in 600-nm diameter vesicles. After acid catalyzed transesterification of the lipid polymers, the derived acyl chain polymers were examined by SEC. These polymers have a common prominent peak corresponding to a polystyrene equivalent molecular weight between 2.5×10^4 and 2.2×10^3. Size exclusion chromatography (SEC) and chemical ionization mass spectrometry revealed the presence of the polymerized acyl-chain dimer and trimer after methanolysis of the lipid polymer.

Introduction

Much effort has been extended to the stabilization of vesicle membranes so that they may be studied as models of biological membranes, or as useful agents in technological applications, such as drug delivery and controlled release.[1,2] Several methods have been used to stabilize the lipid bilayer structure in vesicles, such as adding bolaphiles, amphiphilic polymers, and polymerizing the lipids that form the vesicles. Lipids containing styrenes,[3] acrylates,[4] butadienes,[5,6] and thiols[7] have been incorporated into lipids and polymerized in bilayer structures to stabilize vesicles. Our research efforts with polymerizable lipid systems have focused on diacetylene lipids,[8-10] in particular 1,2-bis(tricosa-10,12-dinoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$).[11,12] In addition to being polymerizable, it has been observed that $DC_{8,9}PC$ forms lipid microcylinders or tubules.[13] Among other factors, the vesicle size is reported to affect tubule formation.[14] Large, multilamellar vesicles form tubules when slowly cooled below the phase transition temperature, whereas small unilamellar vesicles form extended sheets when slowly cooled to 0 °C. In this paper we investigated whether the polymerization process was also dependent upon vesicle size.

Diacetylenes polymerize in the solid state by a 1,4 addition mechanism when irradiated by 254-nm ultraviolet (UV) light[15] (Figure 1). Individual $DC_{8,9}PC$ lipids have a diacetylene in both acyl chains. Therefore, each acyl chain of $DC_{8,9}PC$ may polymerize, creating a complex polymer. The lipid polymer formed upon exposure of 254-nm UV irradiation of $DC_{8,9}PC$ dispersions can be described as a copolymer of the polymerized diacetylene acyl chains joined by the lipid's glycerol backbone. Removal of the glycerol backbone from the lipid polymer allows independent characterization of the diacetylene acyl-chain polymer. By resolving these two polymeric forms, we could gain a better understanding of the relationship between vesicle size and the photopolymerization products of $DC_{8,9}$-PC. Such an understanding impacts on many applications utilizing vesicle stabilization, e.g. controlled release. For this investigation, we prepared various populations of vesicles of $DC_{8,9}PC$ with a narrow size dispersity by the extrusion technique, photopolymerized the vesicles, and examined the lipid polymer products by size exclusion chromatography (SEC). SEC has been used to characterize the molecular weight of lipid polymers.[16] Then, the glycerol backbone was removed from the lipid polymer by acid catalyzed transesterification,[17] and the derived diacetylene acyl-chain polymer was also analyzed by SEC. Since determining molecular weights by SEC depends on calibration of the size exclusion column by polymer standards, and molecular weight standards do not exist for diacetylene polymers, SEC was used as a qualitative tool to compare the lipid and acyl-chain polymers from the different size vesicle samples. The reported molecular weights for the lipid and acyl-chain polymers reflect the molecular weights measured using polystyrene standards to calibrate the SEC column.

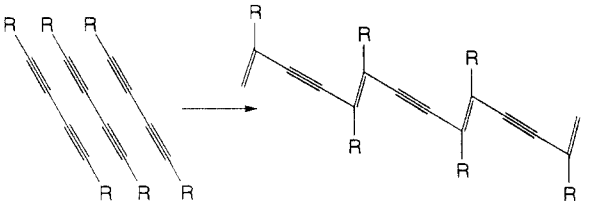

Figure 1. General reaction scheme for the photopolymerization of diacetylenes.

Experimental Section

Materials. 1,2-Bis(tricosa-10,12-dinoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) was synthesized by published procedures[18] and was pure by ^1H NMR and TLC. All solvents were reagent grade or better. HPLC grade chloroform used for size exclusion chromatography was purchased from Burdick and Jackson (Muskegon, MI 49442).

Vesicle Preparation. Solid $DC_{8,9}PC$ (16 mg) was dissolved in a slight excess of HPLC grade chloroform. A lipid film was formed by evaporating the chloroform under a gentle stream of Ar and then dried under vacuum for >15 h. The film was hydrated in deionized water to a final concentration of 2 mg/mL. The suspension was heated to 50 °C in a water bath, vortexed, and

* To whom correspondence should be addressed.
† Center for Biomolecular Science and Engineering.
‡ Chemistry Division.
® Abstract published in *Advance ACS Abstracts*, December 1, 1993.

then frozen in a dry ice/acetone bath. This freeze/thaw cycle was repeated five times.[19] Lipid vesicles were coarsely sized by extrusion through a 0.6-μm Nucleopore filter at 50 °C in a Lipex Extruder (Lipex Biomembranes Inc., Vancouver, BC, Canada V6R 2K2). The temperature of the sample and extruder was maintained at or near 50 °C with a circulating water bath. As reported by Mayer and co-workers, the final vesicle size was reached after the suspension was passed four times through a filter with a pore size near the desired vesicle size.[20] In the case of the 600-nm diameter vesicles, the lipid suspension was not vortexed during the freeze/thaw cycles, and the lipid suspension was passed through the 0.6-μm filter a total of six times. The vesicle suspension was transferred to a quartz reaction chamber maintained at 50 °C. A slow stream of argon was bubbled through the mixture to cause gentle stirring, but not foaming. The vesicle solution was cooled at <0.5 °C/min to 25 °C. After equilibrating at 25 °C for 1–2 h, the vesicle size was determined by light scattering using a Coulter Model N4MD (Coulter Electronic, Inc., Miami Lakes, FL 33014), with the angle of detection at 90°. This general scheme was used to produce vesicles with diameters near 50, 100, 300, and 600 nm.

Polymerization. The stirred $DC_{8,9}PC$ vesicle suspensions were polymerized using 254-nm light from a Rayonette photochemical reactor (Southern New England Ultraviolet Co., Hamden, CT). The distance from the lamps to the center of the reaction vessel was 12 cm. Aliquots (1 mL) were withdrawn after 5 min of UV irradiation. The aliquots were freeze-dried and the polymers stored frozen until needed for chromatographic analysis. The freeze-dried aliquots were resuspended in 1.0 mL of HPLC grade chloroform, and 50-μL aliquots of this solution were used for SEC analysis.

Transesterification. Polymerized samples of solid $DC_{8,9}PC$ polymer from each vesicle size were suspended in 1.0 mL of 1:1 $CHCl_3$/MeOH containing 1 mg/mL of *p*-toluenesulfonic acid and allowed to stand at room temperature for 7 days in the dark (examination of samples after 2 and 4 days under these conditions indicated the reaction had not gone to completion by ^1H NMR). Yellow insoluble lipid polymer was gradually replaced by a white precipitate. This precipitate had an R_f similar to that of an authentic sample of glycerophosphocholine by TLC (*n*-butanol/ethyl acetate/acetic acid/water, 1:1:1:1). The solvent was removed from the reaction mixtures under a gentle stream of argon, and the samples were resuspended in 2 mL of HPLC grade chloroform. The transesterified samples were decanted and the supernatant was used for SEC analysis.

Size Exclusion Chromatography. Size exclusion chromatography (SEC) was performed using an ISCO, Inc. Model 2300 pump, at a flow rate of 1.0 mL/min. Separation of the reaction products was accomplished by passing the samples through an Ultrastyragel Linear column (Millipore Corp., Milford, MA 01757) with an upper exclusion limit of 10^7 MW. Polystyrene standards were used to generate a standard curve to approximate the molecular weight of the lipid and acyl-chain polymers. This is an approximation because the elution volume used to correlate the molecular weight of the lipid and acyl-chain polymers to the polystyrene standard is affected by the conformation the polymers adopt in solution in addition to their molecular weight. The polymer products were detected using an ISCO, Inc. V-4 UV–vis detector, at 280 nm. The average molecular weight of the lipid polymer was calculated using the weighted average of the response at elution volumes 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 mL, and the average molecular weight of the acyl-chain polymer was calculated using the weighted average of the response at elution volumes 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 mL.

Mass Spectrometry. Mass spectra were obtained using a Finnigan TSQ-70 triple quadrupole mass spectrometer. Ions were produced by fast-atom bombardment (FAB) or by desorption chemical ionization (DCI). In the FAB experiments, samples were suspended in a small amount of glycerol and applied to a copper probe tip. The samples were then bombarded with an 8-keV Xe atom beam. In the DCI experiments, samples were applied as chloroform solutions to a wire emitter probe tip and the solvent was allowed to evaporate. Once the probe was introduced into the instrument, a current through the emitter was used to rapidly increase the probe temperature and thermally desorb the sample in the ion source. Ammonia was used as the reagent gas at pressures of 5–6 Torr, which produced abundant protonated or ammoniated species. Tandem mass spectrometry was used to gain some insight into the structure of the protonated molecules. In these experiments, the ion of interest was selected with the first quadrupole and allowed to undergo collisions with Xe in the second (collision cell) quadrupole. Typical Xe pressures were 0.3–0.5 mTorr. Products of the collisions were then analyzed by the third quadrupole.

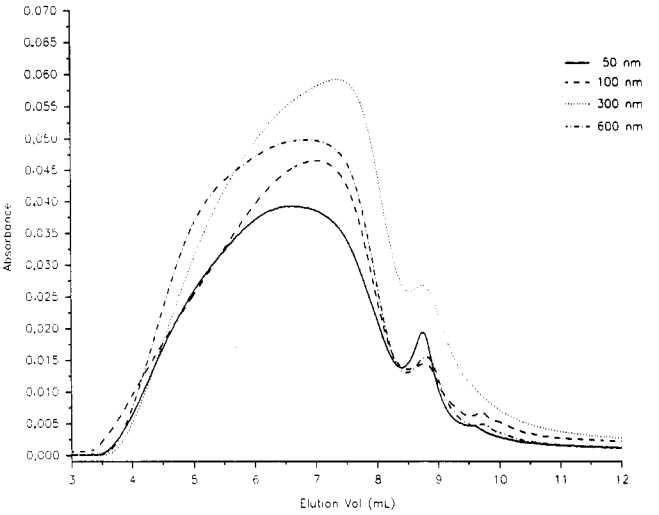

Figure 2. SEC chromatograms of the lipid polymer from each vesicle size after 5 min of UV irradiation.

Table 1. Extruded Vesicle Size (nm) before and after Photopolymerization Measured by Light-Scattering

stated pore size of filter	before photopolymerization	after photopolymerization
50	63 ± 18	68 ± 22
100	95 ● 32	97 ± 35
400	303 ● 37	320 ± 40
600	637 ● 80	685 ● 121

Results

In our hands, vesicles formed at 50 °C and slowly cooled to 4 °C did not maintain their extruded size and morphology. Vesicles in control samples of 50, 100, and 300 nm not irradiated with UV formed large, multilamellar extended sheets[14] and tubules when cooled to 4 °C, while the 600-nm vesicles formed mostly tubules with fewer multilamellar extended sheets. Vesicles in nonirradiated control samples cooled to only 25 °C were stable over the length of the experiment. Therefore, vesicles formed at 50 °C were cooled at a rate of ≤0.5 °C/min and equilibrated at this temperature for 1–2 h before being subjected to UV irradiation. Regardless of vesicle size, the lipid polymer formed under these conditions was pale orange when dispersed in water and was a spongy, deep orange solid after freeze-drying. The size of the extruded vesicles was examined before and after photopolymerization by light-scattering, and the data are compiled in Table 1. The sizes of the vesicles did not change before and after photopolymerization, and these results are consistent with those that have been reported for other polymerizable lipid systems.[3,4]

The SEC chromatograms of the lipid polymers from each vesicle size formed by 5 min of UV irradiation are shown in Figure 2. The total observed molecular weight range for the lipid polymer was between 1×10^7 and 3.4×10^4 for all vesicle sizes. The highest molecular weights observed for the lipid polymers were beyond the linear separation range of the column used. A prominent peak of lipid polymer corresponding to a polystyrene equivalent molecular weight range from 1.6×10^6 to 2.2×10^4 between

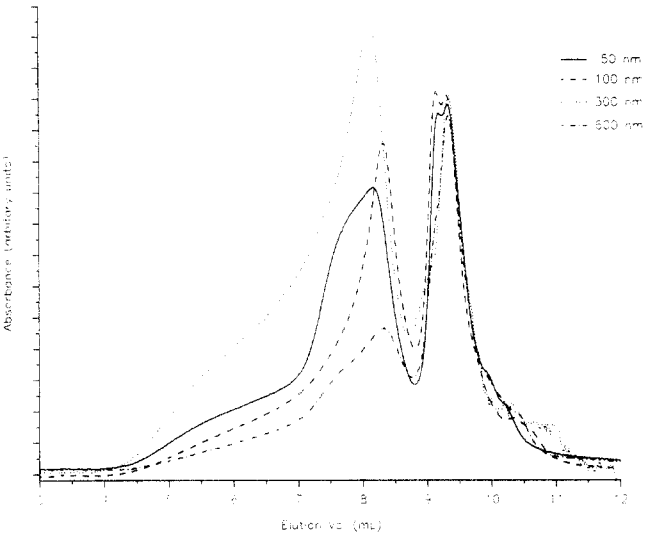

Figure 3. SEC chromatograms of the acyl-chain polymer from each vesicle size after 5 min of UV irradiation and transesterification. The peak heights of the monomeric acyl chain have been normalized to the same height for comparison of the molecular weight distribution.

the elution volumes of 6.2 and 7.8 mL was common to all vesicles sizes. The height of this peak increased with vesicle size from 50- to 300-nm vesicles, but decreased in 600-nm vesicles. However, the sample from 600-nm vesicles had observable amounts of insoluble orange lipid polymer present.

Removing the glycerol backbone from the lipid polymer afforded the diacetylene acyl-chain polymer. SEC analysis of the transesterification products of the lipid polymer using 1 N tetrabutylammonium hydroxide in methanol[21] showed very small oligomeric units, corresponding to dimers, trimers, and a monomeric acyl chain. Examination of the base treated acyl-chain polymer by mass spectrometry did not reveal peaks with masses corresponding to these products.[22] A milder method for dissecting the lipid polymer was provided by acid catalyzed transesterification with *p*-toluenesulfonic acid, which neither altered the UV spectra nor the color of the polymer sample. The acyl-chain polymers were suspended in chloroform. SEC analysis of the acyl-chain polymers from the vesicles was made difficult by the partial insolubility of the acyl-chain polymer obtained from the 600-nm vesicle aliquot and an inability to satisfactorily separate transesterification byproducts. In some of the transesterified aliquots, the acyl-chain polymers were retained on a syringe filter along with the white precipitate (gpc) which formed during the transesterification. Therefore, prior to SEC analysis, the chloroform solution of the aliquots was decanted to remove the insoluble materials settled in the bottom of the sample tubes.

The SEC chromatograms of the transesterified products from each size of photopolymerized vesicles are shown in Figure 3. The peak heights of the monomer have been normalized to directly compare the molecular weight distribution as a function of vesicle size. The molecular weight distribution in the acyl-chain polymers mimic the skewed molecular weight distribution of the lipid polymers. All acyl-chain polymers had a common prominent peak corresponding to a polystyrene equivalent molecular weight range from 2.5×10^4 to 2.2×10^3 eluting between 7.8 and 8.7 mL. This molecular weight range was 10–64 times lower than that observed for the lipid polymers. The 50- and 100-nm vesicles have a much more dimeric acyl chain than that observed from the 300- or 600-nm vesicles.

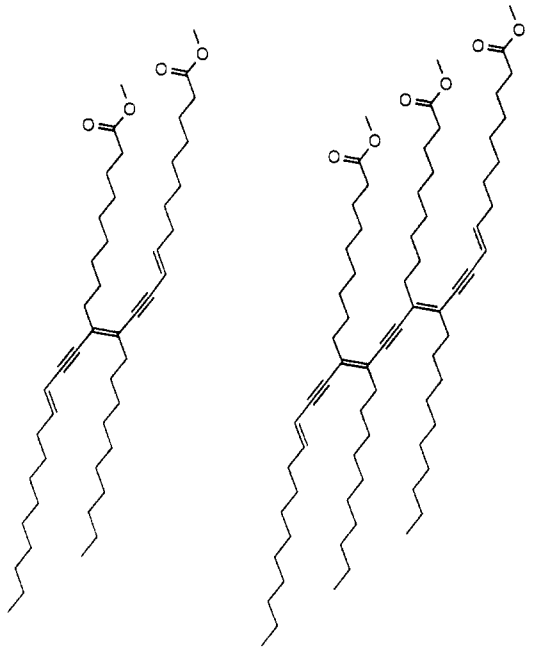

Figure 4. Proposed structure of the methyl ester of the dimer and trimer of the acyl-chain polymer corresponding to the observed masses. The molecular species observed were the ammoniated species.

Using chemical ionization mass spectrometry, the methyl ester of the ammoniated dimer and trimer of the acyl-chain oligomers was identified. The transesterified acyl-chain polymer from 50-nm vesicles showed peaks at m/z = 738 and m/z = 1099. Collection of the fractions having a polystyrene equivalent molecular weight near the weight of the dimer and trimer increased these observed signals in the mass spectrometer. Observation of peaks with larger masses were not possible using this ionization technique. The observed masses and their corresponding proposed structures are shown in Figure 4. To confirm that the observed ions were not proton or ammonia bound aggregates, tandem mass spectrometry was used. Selection of the 738 peak from acyl-chain polymer aliquots followed by collision with Xe, produced a prominent protonated daughter of the dimer, at m/z = 721, corresponding to the *protonated* acyl-chain dimer. The same peak at m/z = 738 was present in the mass spectrum of the methyl ester of the unpolymerized diacetylene acyl chain. However, when selected and passed through Xe, the most prominent daughter had an m/z = 378, which corresponds to the *ammoniated* methyl ester of the diacetylene acyl chain. This observation strongly suggests that the observed dimer in the unpolymerized sample was the ammonia bound dimer, while the dimer from the acyl-chain polymer sample was the covalent dimer product from photopolymerization.

Discussion

Photopolymerization of diacetylenes produces polymers with colors ranging from deep blue to yellow depending upon the length of conjugation in the polymer. Ultraviolet irradiation of 50-, 100-, 300-, and 600-nm synthetic $DC_{8,9}$-PC vesicles at 25 °C produced an orange polymer. Polymerization at 4 °C produced a deep red polymer,[24] usually indicating a higher degree of polymerization. We observed that some of the extruded vesicles of $DC_{8,9}PC$ did not remain as vesicles when cooled to 4 °C. Indeed,

unpolymerized unilamellar vesicles and large multilamellar vesicles have been shown to change morphology when cooled to 4 °C, forming extended sheets and lipid tubules, respectively.[14] Maintaining the dispersion at 25 °C, which is below the chain melting temperature of 43 °C, we were able to carry out the photopolymerization of $DC_{8,9}PC$ *vesicles* in which the acyl chains are in an ordered gel state. This avoided the morphology changes which occur upon cooling to lower temperatures. Interestingly, $DC_{8,9}$-PC suspensions have been observed to contain vesicles with toroidal morphology after 1–10 min of UV irradiation at 25 °C.[25]

Mass spectrometry has provided some structural information about polymerized $DC_{8,9}PC$. Structures which correspond to the observed masses of the acyl-chain dimer and trimer are shown in Figure 4. These structures also correspond to proposed chain termination products for polymerized diacetylenes.[26] Sixl observed that short segments (<6) of reactive oligomeric diacetylenes were butatriene diradicals, while longer oligomers were carbenes. The proposed structures are consistent with a 1,2-hydrogen atom shift at the diradical cumulene or the dicarbene followed by rearrangement to afford the double bond–single bond–triple bond unit. The use of other mass spectrometry techniques such as matrix-assisted laser desorption may provide other useful and insightful information regarding the polymerization reactions of higher mass diacetylene oligomers and polymers.

SEC analysis was used to compare the lipid and acyl-chain polymers from the different sizes of synthetic vesicles. The elution volume used to estimate the molecular weight of the lipid and acyl-chain polymers was based on the elution volumes of polystyrene standards. These observed molecular weight values are affected by the solution conformations the polymers adopt as well as their actual molecular weight. Literature reports have suggested that polydiacetylenes exist as sheets and helices in solution.[27] It seems unlikely that the determined MW using polystyrene standards would correspond to the actual MW of the lipid or acyl-chain polymers. However, the observed apparent MW can be used to compare the relative changes in the lipid and acyl-chain polymers as a function of vesicle size.

The amount of lipid polymer observed by SEC increased with vesicle size for 50- to 300-nm vesicles, but decreased for 600 nm. Although the amount of polymer observed by SEC decreased in 600-nm vesicles, the observed orange insoluble lipid polymer was consistent with a greater degree of polymerization in 600-nm vesicles. The molecular weight distribution of the lipid polymer was skewed to lower molecular weight with respect to a Gaussian distribution expected for a linear polymer sample. This is consistent with a branching polymer since branching reduces the hydrodynamic radius and intrinsic viscosity of a polymer[28] and lowers the apparent molecular weight of a branched polymer relative to a linear polymer with the same number of monomers by SEC.

Transesterification using 1 N tetrabutylammonium hydroxide in methanol[21] was not a useful method for transesterification of the lipid polymer. The base-treated acyl-chain polymer had an altered UV–vis spectrum relative to the lipid polymer, and the SEC chromatogram showed only small oligomers and a monomeric acyl-chain. Acid catalyzed transesterification of the lipid polymer proved to be a more gentle method and allowed characterization of the acyl-chain polymer. SEC of the acyl-chain polymers after 5 min of UV irradiation (Figure 3) showed a common peak in the polystyrene equivalent molecular weight range of 2.5×10^4 to 2.2×10^3 for all vesicle sizes. This peak was 10–64 times lower in observed molecular weight than the prominent peak observed for the lipid polymer, suggesting the glycerol backbone of the lipid joins segments of the polymerized acyl chains, effectively cross-linking the polymer. As noted for the lipid polymers, this peak in the acyl-chain polymers increased with vesicle size from 50 to 300 nm and decreased in the 600-nm vesicles, but this trend was not as strong as was observed for the lipid polymers. The acyl-chain polymer from the 600-nm sample was not completely soluble, but this was consistent with a greater degree of polymerization as vesicle size increased.

Table 2. Comparison of Vesicle Diameter versus the Calculated Area under the SEC Chromatograph for the Lipid and Acyl-Chain Polymers

vesicle diameter by light-scattering (nm)	area of lipid polymer (mm^2)	normalized area of acyl-chain polymer (mm^2)
50	5526	3711
100	6305	3038
300	8001	5757
600	7289	1806

The degree of polymerization in each vesicle size would be difficult to estimate from the SEC chromatograms because the molar absorptivity of the polymer changes with length of the conjugated π system. However, comparison of the SEC chromatograms does allow some qualitative conclusions about vesicle size and the polymerization of $DC_{8,9}PC$ to be made. The integrated areas of observed lipid and acyl-chain polymers from each vesicle size are shown in Table 2. The increase in observed peak area with increasing vesicle size for the lipid polymer indicates that polymerization was more efficient as vesicle size was increased from 50 to 300 nm for the same UV dose. The appearance of solid lipid polymer in the 600-nm vesicle sample was also consistent with this trend. A similar trend, although not as strong, was observed for the acyl-chain polymers from different vesicle sizes. Lopez et al. reported an increased sensitivity of larger vesicles to UV irradiation and argued that the increased order of the lipids in larger vesicles may account for the difference.[29] The fact that the 50- and 100-nm vesicles were observed to have relatively larger amounts of acyl chain dimer is also consistent with increased lipid order in the larger vesicles. Absence of proper alignment between the diacetylene acyl chains of neighboring lipids in the bilayer would result in the termination of the acyl-chain polymer. These data seem to indicate that smaller vesicles have a barrier which hinders the propagation of the *linear* diacetylene acyl-chain polymer. This may not occur for other nonlinear lipid polymers, such as styrenes,[3] acrylates,[4] butadienes,[5,6] and thiols,[7] which have been studied.

The observed acyl-chain dimer could result from an intra- or intermolecular reaction. The acyl chains of PCs extend into the leaflet of a bilayer unequal distances to allow for orderly acyl-chain packing.[30] Thus, the ends of the acyl chains of symmetrically substituted 1,2-diacyl PCs are displaced by 3.7 Å. Indeed, Ohno and co-workers, using butadiene lipids,[5] were able to exploit this difference and polymerize either the sn-1 or sn-2 chains depending on the radical initiator used to start polymerization. Comparison of the photopolymerization products of symmetrical ammonium and phosphonate diacetylene lipids with diacetylene PC lipids indicated that the ammonium and phosphonate lipids were dramatically more efficient at photopolymerization. Lopez and co-workers[29] proposed that only sn-1 to sn-1 and sn-2 to sn-2 photopolymerization was possible and that intramolecular

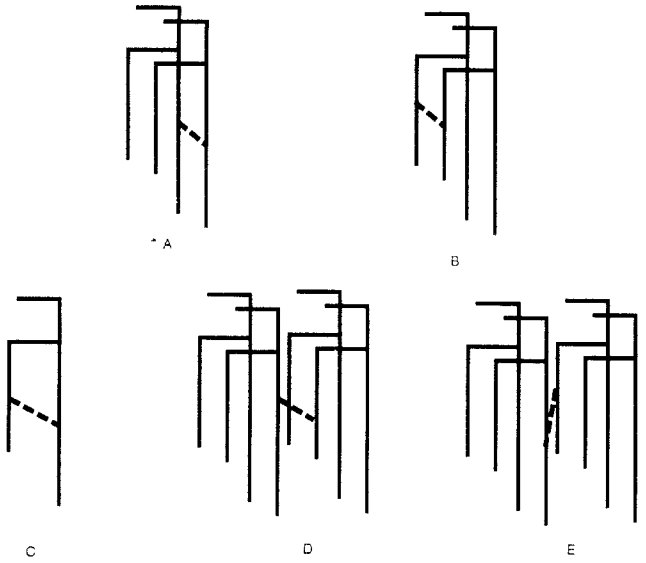

Figure 5. Five models depicting possible reaction pathways for the formation of the dimeric acyl-chain product from the photopolymerization of $DC_{8,9}PC$: (A) reaction between the sn-1 chains of two neighboring lipids; (B) reaction between the sn-2 chains of neighboring lipids; (C) intramolecular rea ction between the sn-1 and sn-2 chains of the same lipid; (D) and (E) reaction between the sn-1 and sn-2 chains of neighboring lipids.

reaction between the sn-1 and sn-2 acyl chains of polymerizable PCs was not possible. However, we believe that if $DC_{8,9}PC$ adopts a conformation similar to other PCs in a bilayer, than displacing the diacetylenes by 3.7 Å and separating them by the 3–4 Å required for hydrocarbon packing would place the diacetylenes in an sn-1 chain approximately 1,4 to the diacetylene in the sn-2 chain of the same or neighboring lipid (Figure 5). If these polymerization pathways are available to $DC_{8,9}PC$ in the bilayer, there would be two types of reaction pathways for polymer product formation. They are represented in Figure 5 and differ according to whether the diacetylene polymer propagates through only the sn-1 or sn-2 diacetylenes or jumps to the plane of diacetylenes in the other acyl chains. For example, if polymerization occurred between only sn-1 acyl chains and only sn-2 acyl chains of two neighboring lipids, the plane of polymer propagation would be maintained (Figure 5A,B, respectively). If the polymer jumps from the sn-1 to sn-2 diacetylene, or vice versa, the plane of polymer propagation changes (Figure 5C–E). Figure 5C shows this intramolecular reaction while parts D and E depict two possible reaction pathways between the sn-1 chain of one lipid and the sn-2 chain of a near neighbor. In models C, D, and E, the alignment needed for chain propagation of the diacetylene chain is lost and, presumably, polymerization could not continue, which represents a chain termination pathway for the photopolymerization of $DC_{8,9}PC$. This jumping between diacetylene planes could explain the large amount of dimeric acyl chain which was observed in the SEC of the samples of transesterified acyl-chain polymer.

The observed photopolymerization behavior of $DC_{8,9}$-PC is consistent with the expected behavior of lipids in a bilayer. In the gel phase of a lipid bilayer, the acyl chains of the lipids are in an extended, all-trans configuration with little rotational freedom. Lateral diffusion of a lipid is slower by 2 or 3 orders of magnitude in the gel phase relative to the melted phase and continues to decrease as the temperature is lowered.[31] Thus, the acyl chains become more ordered as the temperature is lowered. Cooling $DC_{8,9}$-PC vesicles below their phase transition resulted in a change from vesicles to tubules and multilamellar extended sheets. This change in morphology was also accompanied by a change in acyl-chain packing which was highly ordered as observed by FTIR.[32,33] Further, Plant and co-workers found the diffusion of fluorescently-labeled probes in $DC_{8,9}$-PC tubules was too slow to measure.[34] Photopolymerization of $DC_{8,9}PC$ was determined to be most efficient at 4 °C due to the formation of a red polymer,[24] while photopolymerization of vesicles at 25 °C polymerized less efficiently, as indicated by the formation of an orange polymer product. These differences may appear to affect the efficiency of photopolymerization of $DC_{8,9}PC$. The cooler temperature of 4 °C would be expected to slow diffusion of the $DC_{8,9}PC$ relative to 25 °C and increase polymerization efficiency. Diacetylenes are known to polymerize most efficiently in a crystalline state which properly aligns the diacetylenes for 1,4 addition.[35] Tubule and extended sheet structures are highly ordered, and the diacetylenes may be aligned in these morphologies for more efficient polymerization compared to vesicles. Finally, the larger structures of tubules and multilamellar extended sheets may have larger ordered domains compared to the curved vesicles.

The data presented here suggest that there is a relationship between vesicle size and the efficiency of photopolymerization of $DC_{8,9}PC$ vesicles. This was demonstrated by forming vesicles of a known size, irradiating them with UV light, and examining the afforded polymer by SEC. As vesicle size increased from 50 to 300 nm, the integrated area of lipid polymer increased by SEC, while it decreased for 600-nm vesicles. The sample from 600-nm vesicles had insoluble material which is consistent with more efficient polymerization in the 600-nm vesicles. The described methods could also be applied to other polymeric lipid systems to more accurately access the polymer's contribution to the vesicle's properties.

Acknowledgment. We thank Dr. David C. Turner for his valuable comments through the course of this research. This work was supported by the NRL Core Program in Molecular Design of Microstructures. B.M.P. was supported by a National Research Council Postdoctoral fellowship.

References and Notes

(1) Ringsdorf, H.; Berhard, S.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113–158 and references cited therein.
(2) Blume, A. *Chem. Phys. Lipids* **1991**, *57*, 253–273.
(3) Reed, W.; Guterman, L.; Tundo, P.; Fendler, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 1897.
(4) Regen, S. L.; Singh, A.; Oehme, G.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 791.
(5) Ohno, H.; Yoshitaka, O.; Tsuchida, E. *Macromolecules* **1987**, *20*, 929.
(6) Lamparski, H.; Liman, U.; Barry, J. A.; Frankel, D. A.; Ramaswami, V.; Brown, M. F.; O'Brien, D. F. *Biochemistry* **1991**, *31*, 685.
(7) Stefely, J.; Markowitz, M. A.; Regen, S. L. *J. Am. Chem. Soc.* **1988**, *110*, 7463.
(8) Rudolph, A. S.; Singh, B. P.; Singh, A.; Burke, T. G. *Biochim. Biophys. Acta* **1988**, *943*, 454.
(9) Schoen, P. E.; Yager, P.; Sheridan, J. P.; Price, R.; Schnur, J. M.; Singh, A.; Rhodes, D. G.; Blechner, S. L. *Mol. Cryst. Liq. Cryst.* **1987**, *153*, 357.
(10) Georger, J. H.; Singh, A.; Price, R. R.; Schnur, J. M.; Yager, P.; Schoen, P. E. *J. Am. Chem. Soc.* **1987**, *109*, 6169.
(11) Singh, A.; Thompson, R. B.; Schnur, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 2785.
(12) Ahl, P. L.; Price, R.; Smuda, J.; Gaber, B. P.; Singh, A. *Biochim. Biophys. Acta* **1990**, *1028*, 141.
(13) Yager, P.; Schoen, P. E. *Mol. Cryst. Liq. Cryst.* **1984**, *106*, 371.
(14) Burke, T. G.; Rudolph, A. S.; Price, R. R.; Sheridan, J. P.; Dalziel, A. W.; Singh, A.; Schoen, P. E. *Chem. Phys. Lipids* **1988**, *48*, 215.

(15) Wegner, G. In *Chemistry and Physics of One-Dimensional Metals*; Keller, H. J., Ed.; 1977; pp 297–314.
(16) Dorn, K.; Patton, E. V.; Klingbiel, R. T.; O'Brien, D. F.; Ringsdorf, H. *Makromol. Chem., Rapid Commun.* **1983**, *4*, 513.
(17) Serrano, J.; Mucino, S.; Millan, S.; Reynoso, R.; Fucugauchi, L. A.; Reed, W.; Nome, F.; Tundo, P.; Fendler, J. H. *Macromolecules* **1985**, *18*, 1999.
(18) Singh, A. *J. Lipid Res.* **1990**, *31*, 1522.
(19) Mayer, L. D.; Hope, M. J.; Cullis, P. R. *Biochim. Biophys. Acta* **1986**, *858*, 161.
(20) Mayer, L. D.; Hope, M. J.; Cullis, P. R.; Janoff, A. S. *Biochim. Biophys. Acta* **1985**, *812*, 55.
(21) Brockerhoff, H.; Yurkowski, M. *Can. J. Biochem.* **1965**, *43*, 1777.
(22) By mass spectrometry the most prominent peak was at m/z = 424, which corresponds to the protonated Favorskii–Shostakovskii reaction product[23] of the diacetylene acyl chain. A structure for this product corresponding to the observed mass would be $CH_3(CH_2)_9(CH_3O)C{=}CH{-}CH{=}C(CH_3O)(CH_2)_8{-}CO_2CH_3$. This product was not observed by ^1H NMR or mass spectrometry when the transesterification reaction was performed under analogous conditions with monomeric $DC_{8,9}PC$.
(23) Shostakovskii, M. F.; Trofimov, B. A.; Atavin, A. S.; Lavrov, V. I. *Russ. Chem. Rev.* **1968**, *37* (11), 907.
(24) Johnston, D. A.; McLean, L. R.; Whittam, M. A.; Clark, A. D.; Chapman, D. *Biochemistry* **1981**, *22*, 3194.
(25) Mutz, M.; Bensimon, D. *Phys. Rev. A* **1991**, *43*, 4525.
(26) Sixl, H. *Adv. Polym. Sci.* **1984**, *63*, 51.
(27) Wenz, G.; Miller, M. A.; Schmidt, M.; Wegner, G. *Macromolecules* **1894**, *17*, 837.
(28) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size-Exclusion Liquid Chromatography*; John Wiley and Sons: New York, 1979.
(29) Lopez, E.; O'Brien, D. F.; Whitesides T. H. *J. Am. Chem. Soc.* **1982**, *104*, 305.
(30) Pearson, R. H.; Pascher, I. *Nature* **1979**, *281*, 499.
(31) Small, D. M. *The Handbook of Lipid Research: The Physical Chemistry of Lipids*; Plenum Press: New York; 1986; pp 528–531.
(32) Caffrey, M.; Hogan, J.; Rudolph, A. S. *Biochemistry* **1991**, *30*, 2134.
(33) Rudolph, A. S.; Burke, T. G. *Biochim. Biophys. Acta* **1987**, *902*, 349.
(34) Plant, A. L.; Benson, D. M.; Trusty, G. L. *Biophys. J.* **1990**, *57*, 925.
(35) Patel, G. N.; Chance, R. R.; Witt, J. D. *J. Chem. Phys.* **1979**, *70*, 4387.