

## Selective Polymerization of Double-Diene Lipid Assemblies: a Novel Approach to Ladder-Like Polymers

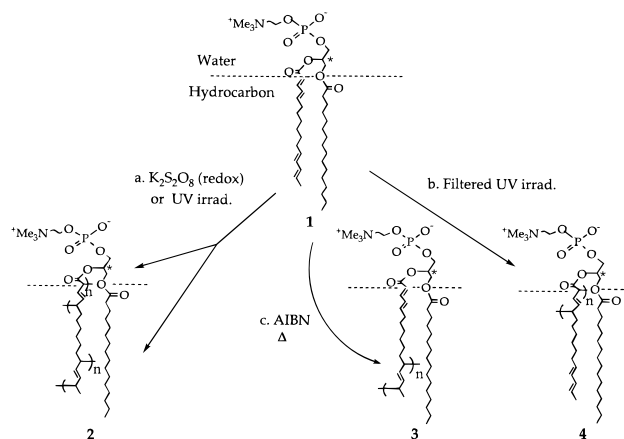
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The polymerization of supramolecular assemblies is a notable method to modify the chemical and physical properties of the assembly. On the other hand, the polymerization process and the polymers formed are dependent on the assembly. The reactivity of polymerizable amphiphilic monomers in two-dimensional supramolecular assemblies, such as hydrated lipid bilayers, is dependent on the mode of initiation, the polymerizable group, and the position of the reactive group in the amphiphile.<sup>1</sup> Unlike monomers in isotropic media, the motion and orientation of hydrated amphiphilic monomers is controlled by the local environment of the assembly. Recent studies have advanced the understanding of polymerization in the constrained environment of lipid bilayers. Systematic investigations of the linear polymerization of reactive lipids in bilayers reveal that at high conversions the polymer chains are likely to be terminated by reaction with initiator fragments (i.e., primary termination).<sup>2–5</sup> Relatively large degrees of polymerization were observed for the radical polymerizations of acryloyl-, methacryloyl-, and sorbyl- substituted lipids.<sup>3,5–7</sup> Polymerization in lipid assemblies proceeds in a linear or cross-linking manner depending on the number of polymerizable groups in the monomeric lipid.<sup>8–10</sup> Lipids that contain a single reactive moiety in either of the hydrophobic tails or associated with the hydrophilic head group yield linear polymers. The polymerization of lipids with reactive groups in each hydrophobic tail yields cross-linked polymers. These results do not indicate if the cross-linking is simply a consequence of two reactive groups per lipid or also depends on their location in each lipid. In order to examine this question, we have designed a polymerizable lipid with two reactive groups in the same lipid tail. In principle, the polymerization of such a molecule in an organized assembly could yield either cross-linked or ladder-like polymers.

The lipid chosen for these initial studies was 1-palmitoyl-2-(2,4,12,14-tetraenepalmitoyl)phosphatidylcholine (**1**) that contains two polymerizable diene groups in the *sn*-2-acyl chain. This double-diene lipid was obtained from the acylation of 1-palmitoyl-2-hydroxy-*sn*-phosphatidylcholine with 2,4,12,14-tetraenepalmitic acid.<sup>11</sup> One group (i.e., the dienoyl) is conjugated with the ester carbonyl of the fatty acid chain, and the other is a disubstituted 1,3-diene. The UV spectrum of the hydrated lipid shows diene and dienoyl absorption peaks at a  $\lambda_{\text{max}}$  of 230 nm ( $1.2 \times 10^4$ ) and 260 nm ( $1.2 \times 10^4$ ), respectively. The selective polymerization of these two reactive groups is also possible because they exist in regions of different polarity. Ohno et al. showed that the position of the polymerizable group in a phospholipid governs the type of radical



**Figure 1.** Simultaneous (a) and selective (b,c) polymerization of bilayers of lipid **1**.

initiation that can be usefully employed.<sup>12</sup> The polymerization of a dienoyl group in the *sn*-1-chain of 1,2-bis(2,4-octadecadienoyl)-*sn*-phosphatidylcholine could be initiated with the hydrophobic AIBN (2,2'-azobis(isobutyronitrile)), whereas a dienoyl group in the *sn*-2-chain could only be initiated with the water soluble AAPD. Here we report the selective polymerization of **1** and characterize the nature of the poly-**1**. The differences in physical properties of cross-linked assemblies vs those composed of linear polymers permit identification of the type of polymerization that occurred (i.e., cross-linked lipid vesicles have greater colloidal stability to added surfactants, lower bilayer permeability to small molecules, and the dried cross-linked lipid is insoluble in organic solvents).<sup>9,10,13–15</sup>

Lipid **1** was hydrated (200  $\mu\text{M}$ ) and then extruded to give 100 nm diameter unilamellar vesicles. The polymerizations described below did not alter the measured vesicle size. The simultaneous polymerization of both the diene and dienoyl groups in **1** to give polymer **2** was accomplished either by unfiltered UV irradiation or by the use of water soluble redox initiators that yield the membrane permeable hydroxyl radical (Figure 1). The selective photopolymerization of the dienoyl group resulted from exposure to filtered light from a high-pressure Hg/Xe lamp. In contrast, the selective polymerization of diene was achieved with hydrophobic radicals from AIBN. The linear polymer **3** produced from the AIBN initiation of the diene had a relative number average degree of polymerization of 350 with a polydispersity of 2.<sup>16</sup> These experiments show that in vesicles of **1** the diene group located near the terminus of *sn*-2-tail is in a hydrophobic environment, whereas the dienoyl group is accessible to hydrophilic initiators.

The selective polymerization of either group can be used in tandem for the sequential polymerization of bilayers of **1**. If

(11) The 2,4,12,14-tetraenepalmitic acid was accessible in three steps from commercially available (*E,E*)-8,10-dodecadien-1-ol, which was oxidized to the corresponding aldehyde using pyridinium dichromate in  $\text{CH}_2\text{Cl}_2$ . The Wittig–Hörner reaction of this aldehyde and trimethyl 4-phosphonocrotonate gave methyl 2,4,12,14-tetraenepalmitate. The hydrolysis of the methyl ester was catalyzed by 1.5 equiv of KOH in methanol at reflux for 4–5 h to afford the desired fatty acid. The stereochemical purity was determined using  $^1\text{H}$  NMR spectroscopy. Isomerization of the (*E,E*)-dienoyl group at the 12- and 14-positions was not observed during the synthesis. The synthesized dienoyl group at the 2- and 4-position was, however, a 4:1 mixture of the (*E,E*)- and (*E,Z*)-isomers. The two isomers were separated by urea inclusion complexation to provide the pure (*E,E*)-isomer.

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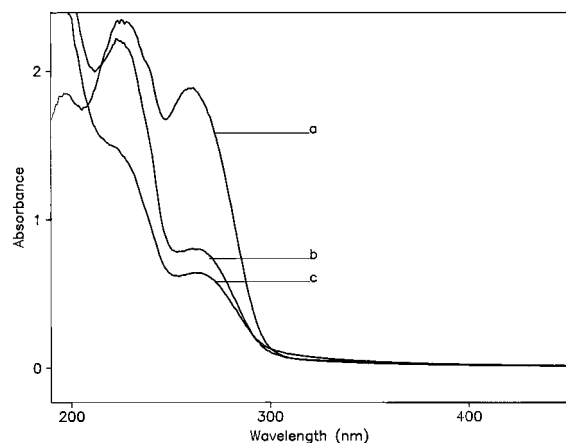
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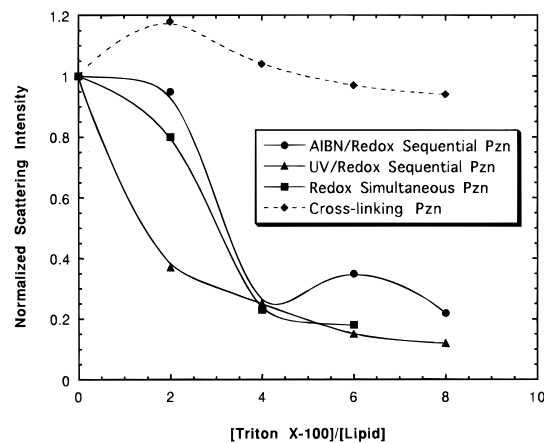


**Figure 2.** The absorption spectra of hydrated bilayer vesicles of **1** before (spectrum a) and after sequential polymerization. Spectrum b shows the absorption after sample exposure to filtered light (Corning CS-056) from a high-pressure Hg/Xe lamp, and spectrum c shows the absorption after redox polymerization of the diene.

the reaction path of the second group to react does not correlate with the reaction path of the first group, then bilayer cross-linking should occur. However, if the second reaction occurs preferentially with an adjoining group in a repeat unit of the polymer formed in the first reaction, then the two reactions should produce a ladder-like polymer without cross-linking. The colloidal stability of the polymerized lipid vesicles to surfactant was employed to determine the nature of the polymers formed from either simultaneous or sequential reaction of bilayers of **1**. Surfactant lysis of vesicles yields mixed micelles that are much smaller than the vesicles, therefore the dissolution of vesicles can be detected by quasi-elastic light scattering. Triton X-100 effectively dissolves unpolymerized and linearly polymerized vesicles to yield micelles, whereas cross-linked vesicles are not disrupted.<sup>15</sup>

Sequential polymerization of **1** was performed in two ways: (1) selective photopolymerization of dienoyl (70% conversion), followed by redox polymerization of the diene (90% conversion) (Figure 2) and (2) selective AIBN polymerization of diene group (95% conversion), followed by redox polymerization of the dienoyl (95% conversion). Persulfate production of hydroxyl radicals was used for simultaneous polymerization. The term simultaneous means the reaction of the diene and dienoyl groups in the bilayer are concurrent, but does not imply that lipid **1** possesses two propagating species at the same time. Polymer **2** obtained from either sequential or simultaneous polymerization were characterized by surfactant lysis of the vesicle. Each of the three modes of polymerization of **1** gave the lipid vesicles that were disrupted by the Triton X-100, showing that the vesicles were not cross-linked (Figure 3). Furthermore the polymers could be isolated from the vesicles, dissolved in organic solvents, and analyzed by NMR, which showed the disappearance of the characteristic dienoyl protons (7.20 ppm) and diene protons (5.60 ppm), respectively.

These results indicate that both the dienoyl and diene groups in **1** preferentially react with same neighbor lipid. An alternative interpretation that their reactions occur in nonoverlapping domains of the bilayer is excluded by the high conversion to polymer. Therefore, the preferred mode of polymerization produces ladder-like polymers. Since the degree of polymerization of the poly(diene) and poly(dienoyl) are unlikely to be the same, the polymers produced from **1** are not true ladder polymers. This is because the two polymer chains are not identical in length, rather one side is likely to be composed of a long continuous polymer and the other consists of shorter polymer lengths. Ladder polymers are of particular interest since they are more resistant to all types of degradation



**Figure 3.** Test of the stability of polymerized vesicles to surfactant (TX-100). The normalized light-scattering intensity of various polymerized vesicles is shown as a function of the molar ratio of TX-100 to lipid. The solid curves connect the data points from vesicles of **1** that have been polymerized either by sequential (AIBN then redox or filtered UV then redox) or simultaneous procedures. The dashed curve is data from a cross-linked vesicle that is shown for comparison. The cross-linked vesicle was prepared from of a phosphatidylcholine with a sorbyl group at the end of each lipid tail.<sup>5</sup>

compared to conventional polymers.<sup>17,18</sup> Many regular ladder polymers have been utilized as liquid crystalline materials due to their stiffness and restriction to rotation. Cyclization and 1,3-dipolar addition reactions are frequently employed to yield ladder polymers. The ladder-like polymers formed from bilayers of **1** are distinctive because of the relatively long aliphatic link between the two polymer chains. In the present case, the spacer link between the two reactive moieties is six methylenes. At some separation distance, the congruence of the preferred reactivity of the two groups will diminish to the point that the polymerizable groups will react independently, affording cross-linked polymer instead of the ladder-like polymers. This situation has been demonstrated in solid phase polymerizations.<sup>19,20</sup> Independent reaction of acrylate and cyano groups separated by a spacer of 13 atoms afforded a cross-linked two-dimensional polymer. Studies of the polymerization of homologs of **1**, where the spacer length is systematically varied, are expected provide further insight into this critical feature of polymerizations in organized media.

These initial results demonstrate some important aspects of bilayer polymerizations that could be usefully employed to prepare novel polymer architectures. Lipid **1** is asymmetric in nature as one end is hydrophilic and the other is hydrophobic. Upon polymerization of **1** in constrained two-dimensional bilayers, the ladder-like polymers obtained have different functionalities at each side of the ladder structure, in a manner that would be difficult to achieve by solution polymerization. The double-diene structure of **1** or similar molecules can be modified to substitute other reactive groups at either or both sides of the ladder-like polymer. Thus, the chemistry described here offers the possibility of post-polymerization chemical modification of asymmetric ladder-like polymers in order to spatially organize entities for various materials objectives.

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