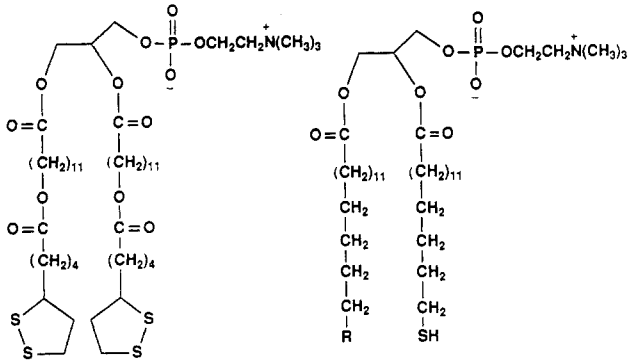
Communications to the Editor

On/Off Switching of Liposome Polymerization by pH Adjustment

In this report we describe the use of 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (I), 1,2-bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (II), and 1-palmitoyl-2-(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (III) in producing liposomes that can be polymerized, or made to lie in a dormant state, by pH adjustment. The ability to "switch on" and "switch off" this polymerization provides a novel method for obtaining a *continuum of polymeric/monomeric states within the same vesicle*. The mildness of the conditions that are required to carry out this polymerization, together with the ability to control the extent of polymer formation within the bilayer, should make these lipids particularly attractive for constructing a wide variety of biomembrane models and devices.[2]

We have previously reported the synthesis of 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (I) and have shown that it can be polymerized in vesicular



I

II, R= SH
III, R= H

form by treatment with dithiothreitol under basic conditions.[3,4] We have also shown that polymerized vesicles can be prepared from 1,2-bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (II) through an oxidative coupling reaction.[5] Here we describe the use of II, and its monothiol analogue (III),[6] as latent nucleophiles that initiate the ring-opening polymerization of I (via thiolate–disulfide interchange). We further show that the polymerization process can be stopped and reinitiated via modest changes in pH.

Rapid injection of 90 μL of a 20 mM ethanolic solution of a 95/5 mixture of I/II into 0.75 mL of 10 mM borate buffer (140 mM NaCl, 2 mM NaN_3, pH 6.4)[7] produced small unilamellar vesicles having an average diameter ranging between 350 and 450 Å (dynamic light scattering). Analysis of the dispersion by thin-layer chromatography and by UV showed that no polymerization occurred after 12 h at 23 °C; i.e., only starting monomer was detected. Upon raising the pH to 8.4, a ring-opening polymerization ensued. Thin-layer chromatography indicated that the polymerization was essentially complete after 4 h;[3] only a polymeric product was observed at the origin. The extent of polymerization was confirmed by following the disappearance of the 1,2-dithiolane moiety at 333 nm (Figure 1). Examination of the dispersion by dynamic light scattering showed that the size and size distribution of the liposomes were unaltered upon polymerization.

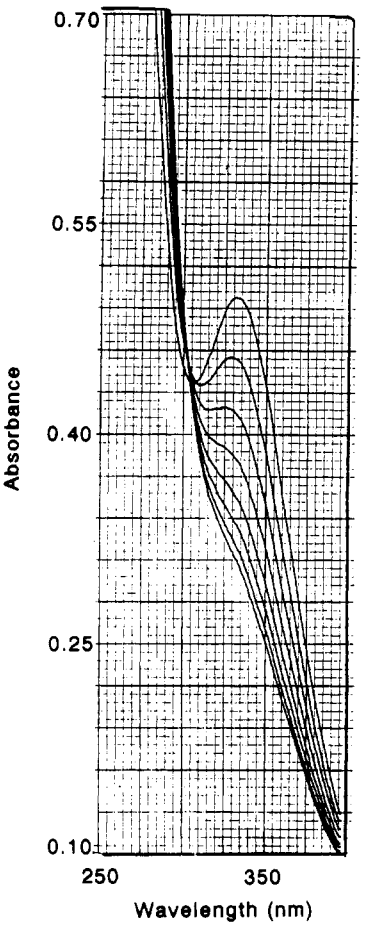


Figure 1. Disappearance of the 1,2-dithiolane moiety (333 nm) within ethanol-injected vesicles derived from a 95/5 mixture of I/II at pH 8.4 (23 °C) as a function of time. The extent of ring opening was determined after 0, 20, 40, 60, 80, 100, 120, and 140 min.

The decrease in absorbance of the 1,2-dithiolane moiety obeyed clean pseudo-first-order kinetics for more than 4 half-lives. In addition, the rate of polymerization was found to be directly proportional to the mole fraction of the initiator that was present (Table I).[3] The fact that a decrease in pH from 8.4 to 7.4 afforded a decrease in the rate of ring opening by ca. 1 order of magnitude implies that it is only the deprotonated form of the thiol (i.e., the thiolate anion) that is the catalytically competent species. On the basis of analogy to thiol–disulfide interchange reactions that have been extensively investigated in solution, we presume that ring opening proceeds via nucleophilic attack by a growing polymeric thiolate anion on the sulfur atom of a neighboring disulfide moiety.[8,9]

Polymerization of a liposomal dispersion, derived from a 95/5 mixture of I/II, was initiated at pH 8.4 and then "switched off" after 23% conversion by adjusting the pH to a value of 6.4. No further loss of 1,2-dithiolane was noted after 15 min (Figure 2). The polymerization could

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Table I
pH-Triggered Polymerization of Liposomes[a]

pH	temp, °C	initiator	initiator, mol %	half-life,[b] min
8.4	23	II	1	330
8.4	23	II	2	150
8.4	23	II	5	44[c]
8.4	23	II	10	27
7.4	23	II	5	590
7.4	37	II	5	64
8.4	23	III	5	140[d]
8.4	23	III	10	78
7.4	23	III	5	1300
7.4	23	III	10	560

[a] Vesicles were produced via ethanol injection. All reactions were monitored by the disappearance of 1,2-dithiolane and were followed for at least 4 half-lives. [b] Unless noted otherwise, half-lives were obtained from single kinetic runs. [c] Average of three independent experiments (±4 min; 1 standard deviation). [d] Average of two independent experiments (±6 min; 1 standard deviation).

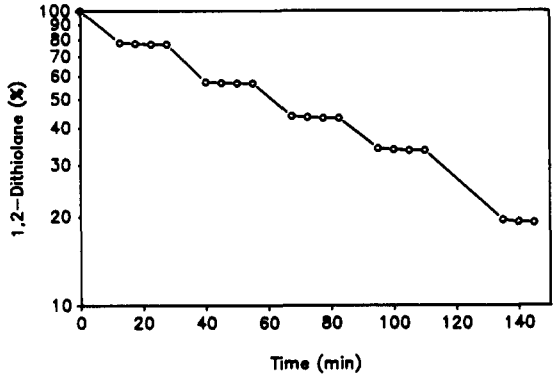

Figure 2. Semilogarithmic plot of the percent of 1,2-dithiolane remaining as a function of time for ethanol-injected vesicles derived from a 95/5 mixture of I/II at 23 °C. On/off cycles were performed by successive alternation of the pH (8.4/6.4).

then be reinitiated ("switched on") by returning to pH 8.4.[10,11] Additional "on/off" cycles were successfully carried out at 43, 55, 65, and 80% conversion; the half-life for each stage was identical.

Similar to homopolymerized liposomes of I, but unlike homopolymerized liposomes produced from a non-cross-linkable analogue (i.e., 1-palmitoyl-2-[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine),[4] *fully* polymerized liposomes made from a 95/5 mixture of I/II are insoluble in $CHCl_3$ and in $CHCl_3/CH_3OH$ (1/1, v/v). This insolubility, together with the fact that such dispersions can withstand 1% sodium dodecyl sulfate,[12] indicates that the lipid membrane is a *cross-linked* network; i.e., each liposome is comprised of one or more cross-linked parts. Partially polymerized liposomes must contain, in addition, a monomeric phase.

The fact that these ring-opening polymerization reactions proceed rapidly at pH 7.4 (37 °C) means that the "on/off" use of these systems can be maximized only under nonphysiological conditions, where a defined monomer/polymer content can be maintained. Despite this limitation, the ability to construct polymerized vesicles from I/II and I/III under the conditions described herein should make these lipid combinations particularly attractive for those biochemical, analytical, and biological applications that require the use of photochemically and thermally sensitive comembrane or entrapped components.[13,14]

References and Notes

(1) Supported by the National Science Foundation (Grant CHE-8703780).
(2) For reviews on polymerized vesicles, see: Regen, S. L. In *Liposomes: From Biophysics To Therapeutics*; Ostro, M. J., Ed.; Marcel Dekker: New York, 1987; p 73 and refs 10–26 cited therein. O'Brien, D. F.; Klingbiel, R. T.; Specht, D. P.; Thyminski, P. N. *Ann. N.Y. Acad. Sci.* **1985**, *446*, 282. Bader, H.; Dorn, K.; Hashimoto, K.; Hupfer, B.; Petropoulos, J. H.; Ringsdorf, H.; Sumimoto, H. In *Polymeric Membranes*; Gordon, M., Ed.; Springer-Verlag: Berlin, 1985; p 1. Johnston, D. S.; Chapman, D. In *Liposome Technology*; Gregoriadis, G., Ed.; CRC Press, Inc.: Boca Raton, FL, 1984; Vol. 1, p 123. Fendler, J. H. *Science* **1984**, *223*, 888.
(3) Sadownik, A.; Stefely, J.; Regen, S. L. *J. Am. Chem. Soc.* **1986**, *108*, 7789.
(4) Stefely, J.; Markowitz, M. A.; Regen, S. L. *J. Am. Chem. Soc.* **1988**, *110*, 7463.
(5) Samuel, N. K. P.; Singh, M.; Yamaguchi, K.; Regen, S. L. *J. Am. Chem. Soc.* **1985**, *107*, 42.
(6) Coyle, L. C.; Danilov, Y. N.; Juliano, R. L.; Regen, S. L. *Chem. Mater.* **1989**, *1*, 606.
(7) The buffer and resulting liposomal dispersion were extensively deoxygenated with nitrogen.
(8) Singh, R.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6304 and references cited therein.
(9) The precise location where the initiator becomes deprotonated, and where ring opening takes place, remains to be established.
(10) In all cases, the pH was adjusted by use of 0.1 M HCl and 0.1 M NaOH.
(11) Liposomes that were prepared from pure I showed no evidence of polymerization after 12 h at 23 °C (pH 8.4).
(12) The turbidity of this polymerized dispersion was unchanged upon addition of 1% SDS; under similar conditions, the turbidity of a nonpolymerized analogue was reduced by ca. 75%.
(13) Using procedures similar to those previously described,[4] we have found that a 95/5 molar mixture of I/II can be readily assembled into multilamellar and large unilamellar vesicles and polymerized by pH adjustment with similar rates.
(14) Mixtures of I/II have been stored in dichloromethane (5 mg/mL) at 4 °C without any evidence of decomposition after 1 month.

Yong-Chan Chung and Steven L. Regen*
Department of Chemistry and Zettlemoyer Center for Surface Studies
Lehigh University, Bethlehem, Pennsylvania 18015

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