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7. To compare the relative reduction potentials of the imide carbonyls, **1** and **2** were reacted with Zn/AcOH in ether at room temperature. Phthalimide **2a** was reduce to give **6c**, and partially reduced **6b** was obtained from **2b**. Under the same condition, succinimide and gluterimide derivatives **1** provided dehalogenated products and carbonyl reduction was not observed.
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9. Reactions of **1** and **2a** with tri-*n*-butyltin hydride and AIBN under high dilution condition produced deiodinated reduction products in quantitative yields. Thus, the possibility of radical cyclization was disregarded. For radical cyclization and ring expansion reaction of 2-haloalkyl-1,3-dicarbonyl systems, see: (a) Dowd, P.; Choi, S. C. *J. Am. Chem. Soc.* **1987**, *109*, 6548. (b) Beckwith, A. L. J.; O'Shea, D. M.; Westwood, S. W. *J. Am. Chem. Soc.* **1988**, *110*, 2565.

Permeation Control of Polymerized Liposome

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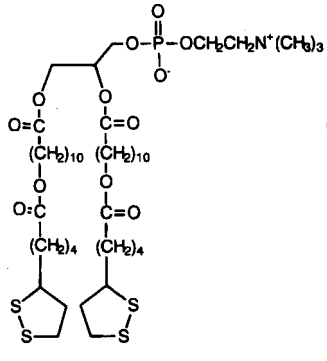
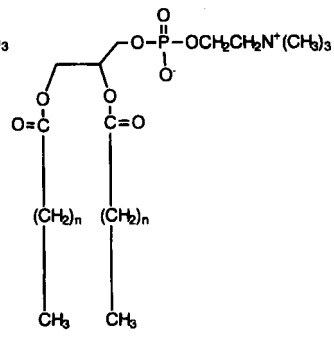
Liposomes are well-known as biomimetic materials applicable to drug delivery,[1] inhibitors of cell adhesion,[2] solar energy conversion, and biomembrane models. Polymerized liposomes have been suggested later as a better choice because of increased stability conferred by cross-linking hydrocarbon chain or head group,[3-5] low probability of lipid exchange and fusion, and the possibility of modifying liposome surface with recognition molecules like antibody, chelating agent.

In this paper we show very simple and easy way of controlling the permeability of our polymerizable lipid (PL). The synthesis of polymerizable phospholipid, 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphorylcholine (**DLL**), was reported elsewhere and synthesized similarly.[6,7]

DLL is superior in drug delivery system to other PL's reported so far in many respects: First, polymerization does not require any harsh conditions like UV light, thermal energy for initiation, and any catalytic materials, mildly proceeding well just by slight pH increase (6.5 to 8.4) in the presence of 5 mol% of cysteine or other thiol-containing material. Second, only 3 hrs of shaking at room temperature is enough for polymerization up to 90%. Third, phospholipid itself is also a major component of biological membrane, and DLL's similar head group structure to natural membrane suggests some biocompatibility. Fourth, the degree of polymerization can be controlled by adjusting pH, and duration of reaction, together with the control of permeability. Fifth, the size of PL can be easily controlled by selecting the appropriate pore size filter before polymerization, and PL can retain the initial size after polymerization for a long period.



DLL



DMPC(n=12), DPPC(n=14)

Among the wide variety of liposome preparation methods, we prefer extrusion to other methods, especially sonication, for the reasons listed below. First, the sonic energy might disrupt the encapsulated biomolecule. Second, SUV (small unilamellar vesicle) prepared by sonication method is quite small for encapsulating drug or vaccine, and has sharper curvature which might give extra strain to the membrane structure, resulting in the loss of flexibility of membrane. Third, sonic method is not good for controlling the size of liposomes, leaving particles coming from the probe.

Accordingly, preparation of PL was done by extrusion method, using extruder from Lipex Biomembrane, Canada. Usually, 3 mg of DLL or DLL with other additives like 1,2-Dipalmitoyl-sn-glycero-3-phosphorylcholine (**DPPC**), and 1,

2-Dimyristyl-sn-glycero-3-phosphorylcholine (**DMPC**) in chloroform solution (total lipid amount: 2.9 μmol) was dried to a lipid film under vacuum, hydrated with 1ml of pH= 6.4 0.02 M phosphate buffer (containing 0.15 mM of carboxyfluorescein, CF) with brief sonication and incubation at 37 °C. The milky solution was extruded 10 times through doubly-stacked 0.4 μm polycarbonate membrane (Costar) at 40 °C under Ar pressure (5 kg/cm^2) to get opalescent mixture. To start polymerization 5 mol% of cysteine was added, and pH was raised to 8.4 by adding an aliquot of 0.3 M NaOH. The mixture was shaken at pH 8.4 overnight at room temperature. Termination of polymerization was confirmed by TLC, because DLL monomer appeared at R_f=0.45 and PL of DLL showed R_f=0 ($CHCl_3$: MeOH, H_2O=65 : 25 : 4). The absorbance of dithiolane ring of the lipid at 333 nm decreased with the progress of ring-opening polymerization. pH was reduced to 6.4 at the end and the non-encapsulated CF was removed by Gel-filtration (Sephadex G-50, 1.5×40 cm), eluting with the same buffer.

From Figure 1, the gel-filtration profile showed that CF was freely leaking through DLL bilayer, resulting in very low encapsulation of CF after gel-filtration. But some PL's with variable ratios of DMPC or DPPC formed tighter bilayer membrane, and decent encapsulation of the CF was observed (The fluorescence intensity was measured by Perkin-Elmer LS-50B Luminescence Spectrometer at λ_{ex}=480 nm, and λ_{em}=518 nm). The ratios of additives were very critical and the tight DPPC or DMPC modified DLL showed very little precipitation after 3-4 days. Among our mixed bilayer liposomes, DLL : DMPC : DPPC=2 : 1 : 1 ratio showed the best performance in tightness, stability, and reproducibility of bilayer membrane. With combined fractions of CF-encapsulated liposomes, we could get the release kinetics of CF at different temperatures. Unlike the non-polymerized liposomes, the polymerized ones are stable in the presence of the surfactants (0.1% solution) like SDS (Sodium Dodecyl Sulfate), Triton X-100, known to disrupt the conventional liposomes.

For the delivery of bigger biomolecules, fluorescein isocyanate dextran (FID, mw=4,400) was encapsulated with DLL alone, showing good encapsulation. The permeation rates of dextran within the PL of DLL demonstrated a big difference from CF which was not even captured with the same liposome. Figure 2 represents one of the FID release kinetic profile with good correlation. In contrast to CF, encapsulation of large molecule was possible without any additives, which might simply the manufacturing procedure, and slower permeation rate would be more preferred for DDS (Drug Delivery System) application. PL of DLL showed no precipitation after 6 months of storage, and the DLL lipid monomer solution in $CHCl_3$ could be stored without any precipitate at 4 °C at least an year (polymerized DLL is not soluble in choloroform).

Table 1. Release Kinetics of Encapsulated Molecule

No	Liposome	Molecules	Temp (°C)	10^9P[d] (cm/min)
1	DMP[a]	CF[b]	25	114
2	DMP	CF	37	163
3	DMP	CF	45	184
4	DLL	FID[c]	25	1.42
5	DLL	FID	37	2.66
6	DLL	FID	45	2.44
7	DLL	BSA	37	No Release

[a] DLL : DMPC : DPPC=2 : 1 : 1 mixture. [b] Carboxyfluorescein. [c] Fluorescein isocyanated dextran. [d] P; Permeation Coefficient. For more information about the calculation of permeation coefficient, see ref 8. The reported values are the average of three independent experiments.

In addition, encapsulated BSA (Bovine Serum Albumin) within the PL of DLL was checked for release kinetics by measuring the retained BSA content from Miller-modified Lowry Assay. No appreciable release of BSA was noticed after 3 days, probably due to the size of bulky BSA (45 kDalton) which could not penetrate DLL bilayer well. Based on all of these results, small molecule can be encapsulated with DLL plus additives, and large one with DLL by itself.

The reason for increased bilayer tightness is not clear at this moment, but probably the rigid structure of PL of DLL which has cross-linked chains at the tail end and kinked ester group in the middle of chain can not form tight bilayer packing enough for holding small CF molecules. With the support from DPPC and DMPC, PL of DLL may fill up the gap among the rigid chains, and the slight difference in chain length between DPPC and DMPC may help in forming tighter bilayer membrane. In mixed bilayer systems, more percentage of DLL will reduce the tightness and more

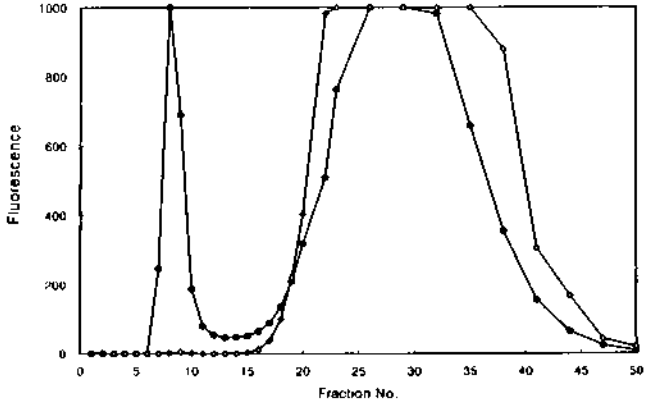


Figure 1. Gel-Filtration Profile of CF-Encapsulated Liposomes. ●: DLL/DMPC/DPPC, ◇: DLL.

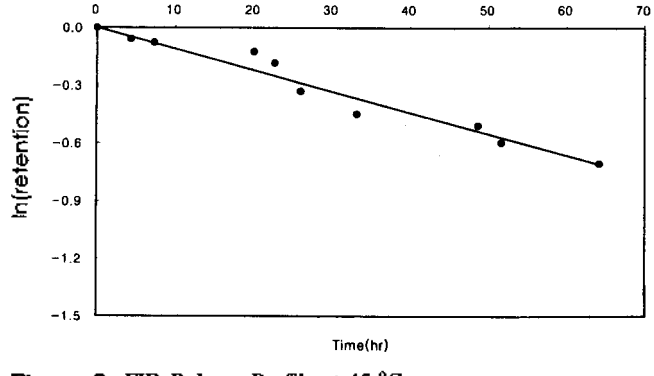


Figure 2. FID Release Profile at 45 °C.

percentage of additives will reduce the stability. Some compromise has been made in formation of both stable and tight bilayer membrane. To explain the optimum ratio (DLL/DPPC/DMPC=2/1/1), more thorough model study remains to be established. Presently we are undergoing projects of controlling permeability of bilayer for large molecules and "smart liposomes" capable of seeking the target site.

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TBSOTf Promoted Conjugate Addition of Enamines, Pyrrole, and Indole to α,β-Enones

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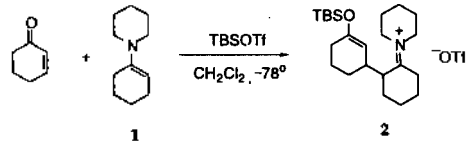
In connection with our research program toward functionalization of α,β-enones via conjugate addition approach,[1-3] we have studied the possibility of TBS triflate promoted conjugated addition of enamines and their related derivatives to α,β-enones. Enamines are normally considered as enol or enolate equivalents and they are often sufficiently reactive to add to conjugate acceptors when heated in solution.[4] If enamines would undergo the conjugate addition reaction to α,β-enones, it is expected that the resulting enolates might be trapped with TMSCl to afford synthetically very useful silyl enol ethers for further α-functionalizations.[5]



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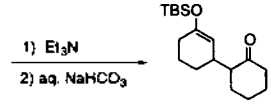

3 (84%) (1)

In order to assess that possibility, we began our studies by mixing 2-cyclohexen-1-one with piperidine enamine of cyclohexanone (**1**) in dichloromethane at room temperature for 24 h. Although the reaction proceeded to some extent, the conjugate addition product **3** was obtained in less than 20% yield. When the same reaction was carried out in toluene at 80 °C for 24 h, only a trace amount of the conjugate addition product was observed. Addition of TMSCl to the reaction mixtuire did not significantly speed up the reaction, indicating that TMSCl was not strong enough to activate the carbonyl group. Therefore, we turned our attention to much stronger reagent TBSOTf as a promoter.[6] When the same reaction was carried out in the presence of TBSOTf in dichloromethane, the reaction proceeded smoothly and was complete almost instantly at − 78 °C, yielding the 1,4-addition product **3** bearing a silyl enol ether group in 84% yield after aqueous workup (eq 1). Evidently, the reaction should proceed via intermediate **2**. The silyl enol ether group can be further utilized for the α-functionalization of α,β-enones.

To determine the scope and limitations of the present method, the reactions were carried out with several structurally different α,β-enones using **1** and piperidine enamine of hydrocinnamaldehyde (**4**) in the presence of TBSOTf in dichloromethane at − 78 °C and experimental results are shown in Table 1. When 2-cyclohexen-1-one was treated with **4** under the similar conditions, 1,4-addition product was isolated in 93% yield. However, when the same reaction was carried out with 2-cyclopenten-1-one, a 69 : 13 mixture of 1, 4- and 1,2-addition product was obtained, whereas 2-methyl-2-cyclopenten-1-one was exclusively converted into 1,4-addition proudct under the similar conditions. Furthermore, 1,4-addition products were obtained exclusively with carvone and 3-methylene-2-norbornanone. However, with acyclic enones as Michael acceptors, a mixture of 1,2- and 1,4-addition products was obtained roughly in an equal ratio. Somewhat higher ratio of 1,4- and 1,2-addition products were obtained with **1**. Thus, 2-cyclopenten-1-one and 2-cyclohexen-1-one gave only 1,4-addtion products in high yield, although 2-cyclohepten-1-one gave a 74 : 7 mixture of 1,4- and 1,2-addition product. With acyclic enones, the ratio