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Polypeptide Liposome by Spontaneous Condensation in Bilayer Membranes

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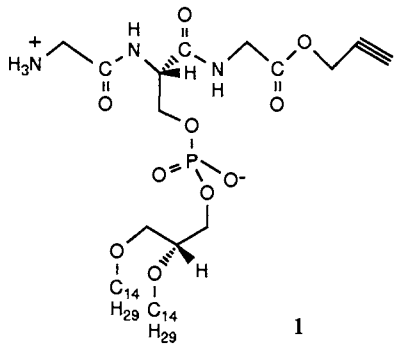
A novel glycerophospholipid having a tripeptide propargyl ester as a head group was synthesized. Polycondensation of the peptide lipid occurred spontaneously in bilayer membranes, resulting in a liposome covered by polypeptide chains consisting of about 20 amino acids.

During the past two decades, liposomes have been extensively investigated as a biomembrane model.[1] Of recent special interests are the synthetic bilayer membranes containing a biomolecular moiety, such as amino acids,[2,3] nucleic acids,[4] or polysaccharides.[5] Several attempts have been made to obtain polypeptide liposomes by condensation of long alkyl α-amino acid methyl esters[6] or amphiphilic amino acids.[7] No complete conversion, however, has been achieved in these systems due to the formation of precipitates. In this letter, the authors wish to report the high degree conversion to the polypeptide liposome by use of spontaneous condensation of the glycerophospholipid derivative.

Glycyl-O-(O-1,2-ditetradecyl-s,n-glycerophospho)-seryl-glycine propargyl ester (**1**) was designed for the present study. Its chiral phosphatidylserine skeleton was chosen for the purpose of close packing of bilayer membranes.[8] Having enough stability for hydrolysis and reactivity for aminolysis in bilayer membranes, a propargyl ester was introduced with a amino group as a reactive group.[8] Phospholipid **1** was synthesized as shown in scheme 1,[9] and a liposomal solution of **1** was prepared by ultrasonication (1 mg/ml in carbonate buffer at pH 8.5) at 0 °C (Branson sonicfier 250, 30 W) for 3 min.



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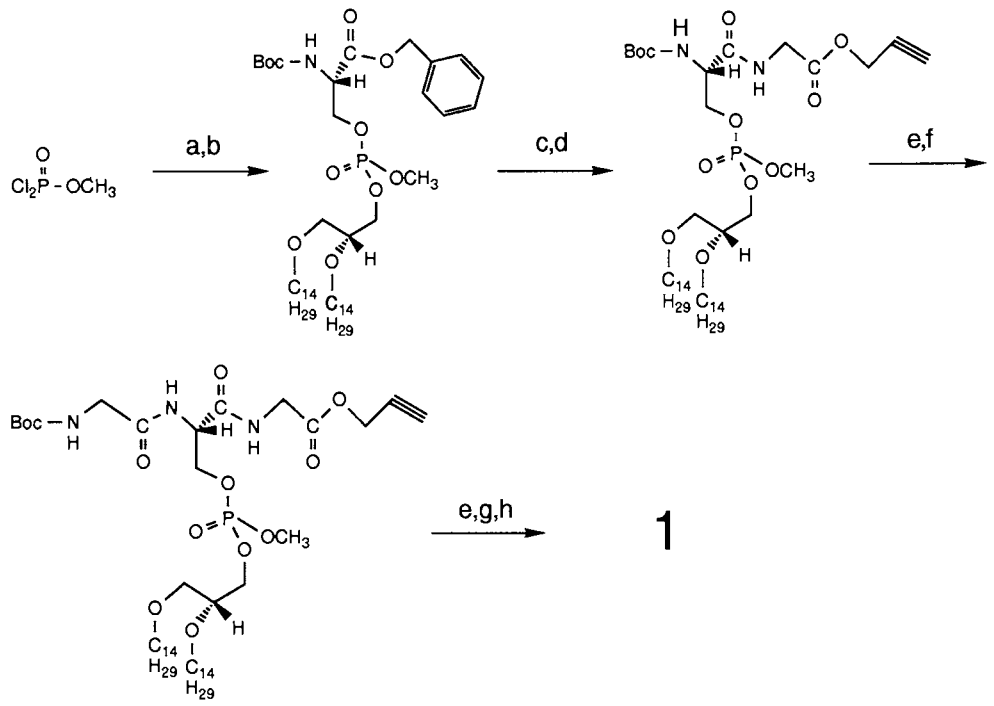

Scheme 1.

a) Boc-L-Ser-OBzl, N-Methylimidazole, THF. b) O-Ditetradecyl-s,n-glycerol, N-Methylimidazole, THF. c) 5% Pd/C, H_2. d) DCC, Glycine propargyl ester, CH_2Cl_2. e) TFA, CH_2Cl_2. f) Boc-Glycine anhydride, Et_3N. f) LiBr, MEK. g) Amberlite® IR-120B. h) Amberlite® IRA-93ZU.

Vesicle formation was confirmed by transmission electron microscopy (TEM, Fig. 1a) and entrapment experiment of the 5 or 6 - carboxyfluorescein aqueous solution. To convert into a polypeptide liposome by intermolecular aminolysis of the propargyl ester, the resulting vesicle solution was kept at 25 °C or 50 °C. IR spectra of **1**[10] before and after the polycondensation in liposome are compared in Fig. 2. The formation of the peptide bond is apparent from the increase of the amide bands (1680 cm^{-1}, 1550 cm^{-1}) with the disappearance of the propargyl ester absorption band (1760 cm^{-1}). Even after the polymerization, the spherical structure of vesicle has still been retained (Fig. 1b) and no precipitation has been recognized. This result indicates that the phospholic ester group has enough hydrophilicity to form the

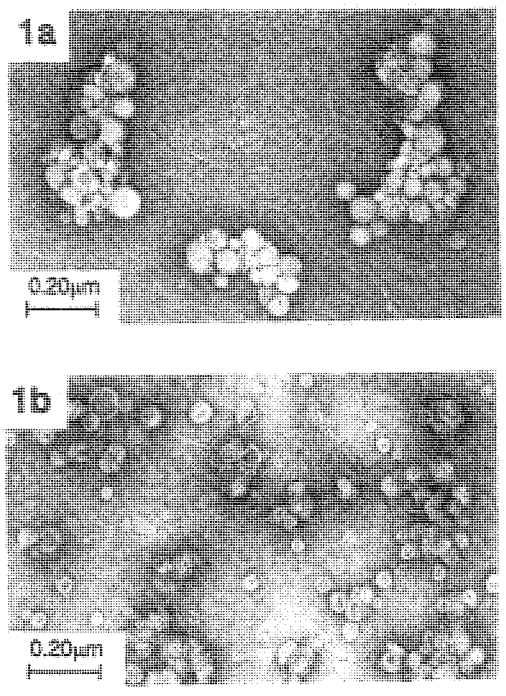

Fig. 1. Transmission electron micrographs of liposomes of **1** before (1a) and after (1b) polycondensation.

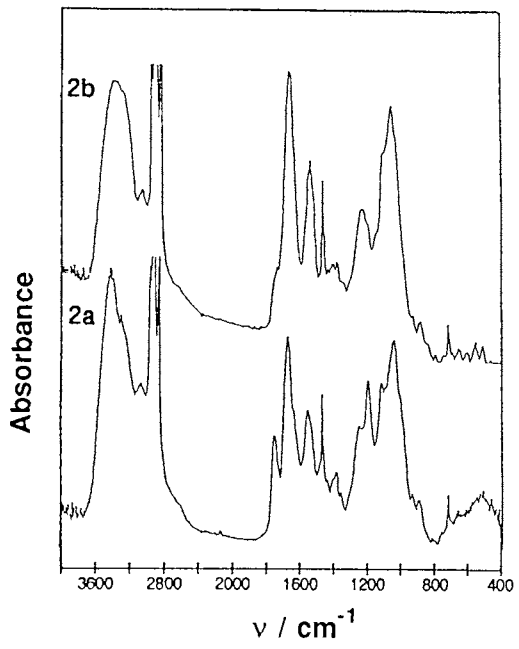

Fig. 2. IR spectra of **1** before (2a) and after (2b) polycondensation at 4 °C for 170 h in the liposome.

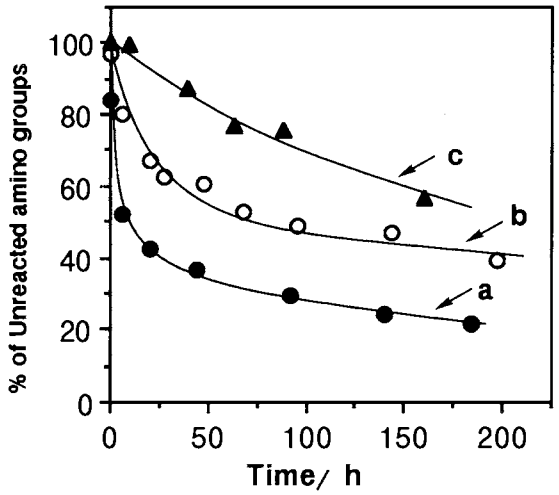

Fig. 3. The plots of the time (h) vs. percent of unreacted amino group of **1** in liposome a) at 50 °C, b) at 25 °C, and c) with Triton X-100 at 50 °C.

bilayer membrane even after the formation of the less hydrophilic polypeptide head group.

Fig. 3 shows the time dependent decrease of the amino group of **1** in bilayer membranes, caused by the aminolysis of **1** for the formation of the polypeptide. The determination of the amino group was carried out by the fluorescamine method.[11] By comparing the curves a and b, one can see that peptide formation occured more effectively at higher temperature. When 10 % of Triton X-100 aqueous solution (100 μl) was added to liposomal suspension (3 ml) in prior to the condensation for the purpose of destruction of the bilayer membranes, the rate of the aminolysis became drastically slow (curve c). This result shows that the ordered bilayer structure is required for the effective formation of the amide bonds.

Degree of the polymerization was determined by the amount of the terminal amino groups of **1** in the liposome. After complete condensation at 50 °C for 260 h, the polymerization degree was estimated to be 6.8, which corresponds to 20 amino acid residues. Molecular weight of the resulting polymer was calculated to be 5400.

In conclusion, the peptide liposome with high polymerization degree was realized by spontaneous condensation of peptidic phospholipid propargyl esters in the liposomal membranes. Further studies on the properties of the peptide liposome are in progress.

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9) Data for **1**. ^{1}H-NMR (400MHz, $CDCl_3$, 318K) δ 0.88 (6H, t, J=7Hz), 1.27 (22H, brs), 1.56 (4H, t, J=7Hz), 2.48 (1H,t, J=2Hz), 3.23-3.50 (3H,m), 3.50-3.64 (2H,m), 3.80-4.42 (4H, m), 4.02-4.08 (2H,m),4.28-4.38 (4H, m+t, J=2Hz); Fab MASS $(M+H)^+$=804; Anal. Calcd for $C_{41}H_{78}N_3O_{10}P_1$: C, 9.77; H, 61.35; N, 5.23%. Found: C, 9.45; H, 61.25; N, 5.00%. Phase-transition temperature, measured by a differential scanning calorimeter (ULVAC DASC-4), was 25 °C.

10) FT-IR spectrum was measured by KBr method after the lyophilized sample was exposed to HCl gas for 5 seconds.

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