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Stabilizing Effect of Diphytanylphosphate on Dipalmitoylphosphatidylcholine Bilayer Membrane

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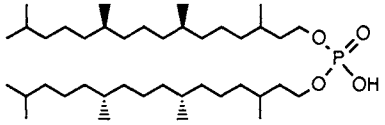
A novel surfactant having two isoprenoid chains, diphytanylphosphate (**1**), was synthesized. Vesicles composed of dipalmitoylphosphatidylcholine (DPPC) and **1** exhibit high barrier effect on the leakage of its contents and resistance to the aggregation.

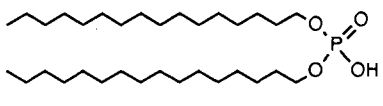
Possible application of liposomes has been extended to various field. The insufficient stability of the bilayer membrane, however, has limited the practical application such as imaging, sensing or drug delivery systems (DDS). To overcome this problem, polymerization of liposomal membranes has been extensively investigated by many groups.[1] Though excellent stable vesicles may be obtained by polymerizing techniques, low biodegradability of the bilayer membrane may cause serious problem especially in medical use.

On the other hand, archaebacteria which inhabit in violent environments such as high temperature and high salt concentration have a extremely stable cell membrane composed of lipids having unusual isoprenoid chains.[2] By use of synthetic lipids, Yamauchi et al. have been reported that the bilayer membranes of isoprenoid lipids show high resistance to the permeability of materials or ions.[3] To obtain a new stabilizer for the bilayer membrane, we designed a novel dialkylphoshate having two isoprenoid chains. In this letter, the authors wish to describe the synthesis and properties of the membrane stabilizer, Di(3RS, 7R, 11R-phytanyl)phosphate (**1**).

As shown in Scheme 1, **1** was synthesized from phytol (**3**) by three steps.[4] 3RS, 7R, 11R-Phytanol (**4**) was prepared by hydrogenation of **3**. After phosphorylation of **4**, resulting **5** was purified by silica gel chromatography (eluent: hexane/ethylacetate=95/5). **1** was obtained by hydrogenolisis of **5** with Adams catalyst (total yield from **3** was 62%).[5]



1



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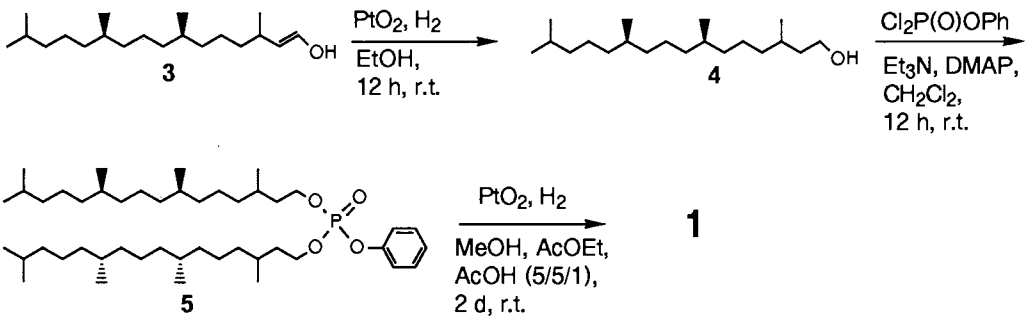

Scheme 1.

The solution of DPPC and **1** (2/1 molar ratio) in chloroform was slowly evaporated at reduced pressure. The vesicles were prepared by stirring the resulting thin film of the mixture in 200 mM 5 (6) carboxyfluorescein (CF) solution of 20 mM Tris-HCl buffer (pH 7.4, 200 mM NaCl) at 40 °C for 30 min. After subsequent sonication (Branson 2000, bath type ultrasonicater) at 25 °C for 30 min, vesicles were sized by extruder (through 0.2 μm pore membrane) at 55 °C. The vesicles were separated from the unentrapped CF by gel permeation chromatography with Sepharose 4B. The formation of vesicles was confirmed by the observation of transmission electron microscopy (TEM).

Figure 1 shows the pressure-area isotherms for monolayers of DPPC, DPPC/**1** (2/1 molar ratio), and **1** on aqueous subphase. The film area of **1** was larger than that of DPPC at every surface pressure (compare with curve a and c). On the basis of the areas of DPPC and **1**, for example, at 40 $mN \cdot m^{-1}$, it is suggested that about half area of the mixed monolayer (DPPC/**1**=2/1) was occupied by **1**. Two destruction pressures were observed at the curve b. This means the phase separation occurs in the mixed monolayer. The behavior of the liposomal membranes must be reflected by this property of monolayer.

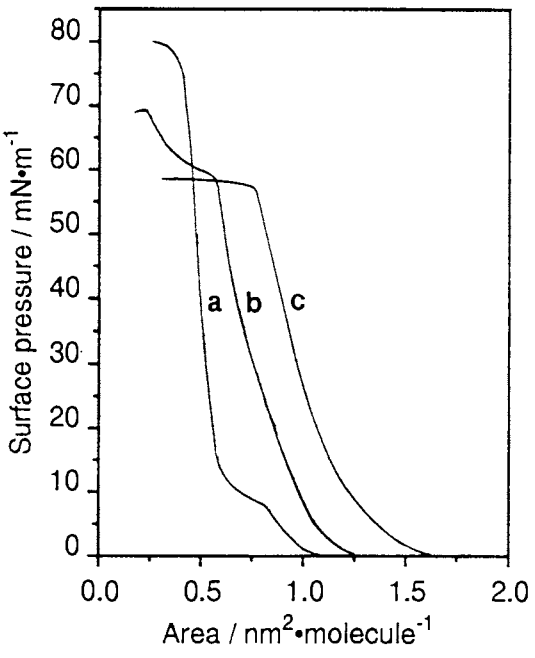

Fig. 1. Surface pressure-area isotherms of DPPC (a), DPPC/**1** (b), and **1** (c) on 20 mM tris-HCl buffer (pH 7.0) at 25 °C.

Figure 2 shows the effects of dialkylphoshates on the leakage of CF from the vesicles at various temperatures.[6] Comparing the plots 2a and 2b, DPPC/**1** (2/1 molar ratio) vesicles exhibited effective

resistance to the leakage of CF than DPPC vesicles.[7] By contrast, such stabilizing effect was not observed at the vesicles composed of DPPC and straight-chain dialkylphosphate **2** (see plots 2c). These results indicate that the bulky structure of isoprenoid chains contributes to high barrier effect on the CF leakage through the mixed bilayer membrane.

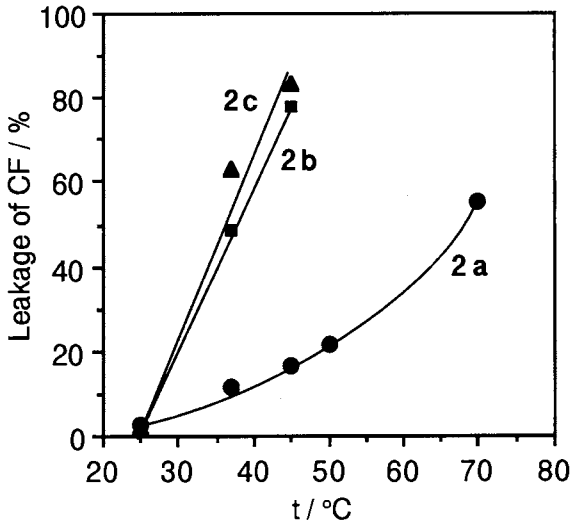

Fig. 2. Temperature dependent leakage of CF from vesicles of DPPC/**1** (2a, ●), DPPC (2b, ■), or DPPC/**2** (2c, ▲) in 60 min at pH 7.4 (20 mM Tri-HCl buffer containing 200 mM NaCl).

Furthermore, **1** can afford negative charge on the membrane surface and avoid the aggregation of the vesicles by electrostatic repulsion. Even DPPC/**1** vesicles were stored at 4 °C in 20 mM Tris-HCl buffer (pH 7.4, 200 mM NaCl) for a month, no aggregate was observed in the suspension. DPPC vesicles, however, aggregated within 2 days.

In conclusion, we demonstrate that the diphytanylphospahte (**1**) having two isoprenoid chains is a potent stabilizer to the bilayer membrane of DPPC. These stable vesicles may be useful for sensing, imaging, or drug delivery systems (DDS). We are now proceeding to investigate their application .

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4) Phytol (**3**) was kindly supplied by Takasago International Corporation.

5) Data for **1**: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.72-0.92 (30H, m), 0.92-1.80 (48H, m), 3.92-4.20 (4H,m); IR (neat), 2960, 2925, 2870, 1460, 1380, 1220, 1190, 1020, 920, 740 cm^{-1}; Fab MASS $(M+H)^+$=659; Anal. Found: C, 71.64; H, 12.47%. Calcd for $C_{40}H_{83}O_4P_1 + 1/2\ H_2O$; C, 71.85; H, 12.57%.

6) The liposomal suspension obtained by the gel permeation chromatography was added to 20 mM Tris-HCl buffer (pH 7.4, 200 mM NaCl) at various temperature instantly, then the fluorescence intensity of CF in outer aqueous phase (minus initial background fluorescence; excitation 492 nm, emission 520 nm) was measured in 60 min. Percent leakage of CF from the vesicles was estimated by the fluorescence intensity, where 100% was the fluorescence measured after the addition of Triton X-100.

7) Similar stabilizing effect was observed at the bilayer membrane comprised of egg-yolk phospatidylcholine and **1** (2/1 molar ratio).

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