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Archaebacterial lipid models: stable liposomes from 1-alkyl-2-phytanyl-*sn*-glycero-3-phosphocholines

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Abstract

1-Alkyl-2-phytanyl-*sn*-glycero-3-phosphocholines (HPhyPC: alkyl = n-$C_{16}H_{33}$; EPhyPC: alkyl = n-$C_{20}H_{41}$), which are artificial chimeric lipids between archaebacterial and ordinary glycerolipids, are prepared. Upon sonication in aqueous media, the lipids gave rise to stable unilamellar liposomes having diameter of 200–1000 Å and gel-to-liquid crystalline phase transition temperature of −11.1°C (EPhyPC) and −10.8°C (HPhyPC). The vesicles were thermally stable (to about 70°C) and retained effficiently 5(6)-carboxyfluorescein and sodium chloride in the aqueous interior. Physicochemical properties of their membranes were discussed in comparison with those of the membranes made of an archaebacterial model lipid, 1,2-diphytanyl-*sn*-glycero-3-phosphocholine.

Keywords: Archaebacterium; Liposome; Membrane; Phytanyl group; FABMS; NMR, ^{1}H-

1. Introduction

Liposomes, lipid vesicles enclosing an aqueous space, have been attracting attention as carriers of bioactive, cosmetic and pharmacological substances such as hormones, enzymes and drugs [1–4]. Although various criteria must be fulfilled to serve as a safe and effective delivery system particularly when applying to living systems [3,4], the vesicles should be stable at least during a period of an application without leaking uselessly the entrapped substances. In this regard, many conventional glycerolipids may not be regarded as perfect liposome-forming materials because their vesicles are apt to release the trapped substances at high temperate and salt conditions [5,6].

On the other hand, various archaebacterial lipids and the model compounds have been examined for liposome preparation. Since archaebacteria habitat hot spring, salt lakes, etc. [7–10], the investigations have perhaps been conducted on the assumption that the plasma membranes might also be tolerant under such extreme conditions. In fact, the liposomes from archaebacterial lipids and model lipids such as DPhyPC [**1**: phytanyl = (3*RS*,7*R*,11*R*)-dihydrophytyl] were very stable to retain proteins, dyes and inorganic salts in the aqueous interior at high temperature (30–70°C) and high salt concentration (0–5 M) [11–15]. However, as discussed below, a problem has been arisen that the archaebacterial lipid-membranes were *slightly* leaky even at ambient temperature. In order to cope with the problem we synthesized a new type of phospholipids, EPhyPC (**2**) and HPhyPC (**3**), and studied the properties of the liposomal membranes. Fig. 1 shows the structures of the phospholipids studied.

2. Materials and methods

DPhyPC and (*RS*)-DPhyPC were prepared according to the previous procedure [13]. DPPC and other known lipids having straight alkyl chains were synthesized by literature methods [16,17]. TLC was carried out on a silica gel-precoated sheet (Merck Art. 5735). Column chromatography was performed using silica gel (Merck 7734, 70–230 mesh). The spots were visualized under ultraviolet light

Abbreviations: CF, 5(6)-carboxyfluorescein; DHPC, 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (palmitoyl = n-$C_{15}H_{31}CO$-); (*S*)-DPhyPC, 1,2-diphytanyl-*sn*-glycero-3-phosphocholine [phytanyl = (3*RS*,7*R*,11*R*)-dihydrophytyl] (see Fig. 1); (*RS*)-DPhyPC, a equimolar mixture of DPhyPC and its *sn*-2,3-diphytanyl isomer (see Fig. 1); EPhyPC, 1-eicosyl-2-phytanyl-*sn*-glycero-3-phosphocholine (eicosyl = n-$C_{20}H_{41}$) (see Fig. 1); FABMS, fast atom bombardment mass spectrum; HPhyPC, 1-hexadecyl-2-phytanyl-*sn*-glycero-3-phosphocholine (see Fig. 1); PC, phosphatidylcholines; R_f, a relative mobility in thin-layer chromatography; T_m, the temperature at midpoint in gel-to-liquid crystalline phase transition; Tris, tris(hydroxymethyl)aminomethane.

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after spraying 0.25 mM aqueous Rhodamine 6G (for the long chain hydrocarbon groups) and the Dittmer–Lester reagent [18]. ^{1}H-NMR spectra were recorded on a Jeol GX-400 spectrometer using a dilute solution in C^2HCl_3 or a mixture of C^2HCl_3 and $C^2H_3O^2H$ (2:1, v/v) and tetramethylsilane as an internal standard. Fast atom bombardment mass spectra (FABMS) were obtained using a Jeol HX-100, whereby a sample was mixed with glycerol on a standard FAB target, then subjected to a beam of xenon atom produced at 8 keV and 2 mA. Ultrasonication was performed by means of a prove-type ultrasonic disintegrator, Ohtake Works, Model 5201. A microprocessor-controlled film balance, Sun-etsu, FDS-20, was utilized for the monolayer studies. Transmission electron micrographs were taken by the use of a Hitachi H-7000 microscope. Other instruments used were a Rigaku DSC-8240 differential scanning calorimeter and a Ohtsuka DLS-700 light scattering spectrometer.

2.1. Synthesis

2.1.1. 1-Eicosyl-3-benzyl-sn-glycerol

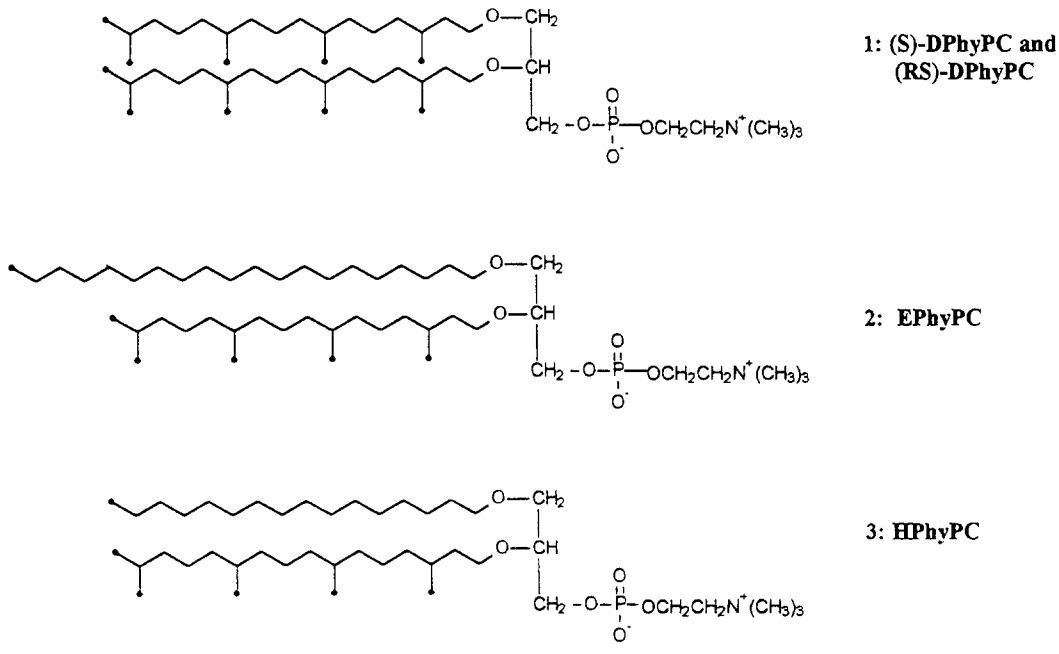
A mixture of 1,2-(dibutyl)stannylene-3-benzyl-*sn*-glycerol (2.1 g, 5.1 mmol) [19] and n-eicosyl bromide (1.9 g, 5.1 mmol) was heated at 150°C for 13 h with stirring magnetically. The cooled reaction mixture was fractionated between chloroform and water. The organic extract was concentrated and applied to a silica gel column. Elution using hexane/ethyl acetate (4:1, v/v) gave 1-eicosyl-3-benzyl-*sn*-glycerol as a colorless solid, which showed a homogeneous spot on TLC; R_f 0.52 (hexane/ethyl acetate (2:1, v/v)); yield 1.0 g (44%); m.p. 40–42°C; $[\alpha]_D^{20}$ +1.5° (c (g/100 ml) 20.8, $CHCl_3$); ^{1}H-NMR (C^2HCl_3): δ 0.86 (t, 3H, ω-CH_3), 1.25 (m, 34H, $(CH_2)_{17}Me$), 4.56 (s, 2H, CH_2-phenyl) and 7.31 (m, 5H, phenyl); FABMS(Xe; matrix, glycerol): *m/z* (relative intensity) 463 ($(M+1)^+$, 25).

2.1.2. 1-Eicosyl-2-phytanyl-3-benzyl-sn-glycerol

1-Eicosyl-3-benzyl-*sn*-glycerol (1.9 g, 4.1 mmol) in tetrahydrofuran (3 ml) was stirred with sodium hydride (0.2 g of 60 wt% dispersion in mineral oil, 5.0 mmol) for 1 h, mixed with (3*RS*,7*R*,11*R*-)phytanyl bromide (1.5 g, 4.1 mmol) and heated by means of an oil bath of 150–160°C for 8 h with stirring magnetically, during which the solvent was allowed to evaporate. The cooled reaction mixture was fractionated between water and chloroform. The organic layer was dried by anhydrous sodium sulfate, concentrated and applied to a silica gel column. Elution with hexane/ethyl acetate (20:1, v/v) afforded a title compound as the viscous oil; 1.6 g (57%); R_f 0.54 (hexane/ethyl acetate (10:1, v/v)); $[\alpha]_D^{20}$ +0.07° (c 40.5, chloroform); ^{1}H-NMR (C^2HCl_3): δ 0.78 (m, 18H, $6CH_3$), 0.89–1.56 (complex m, 61H, $(CH_2)_{18}Me$ and $CH_2CH_2(Me)CH[(CH_2)_3(Me)CH]_3Me$), 4.45 (s, 2H, CH_2-phenyl) and 7.24 (m, 5H, phenyl); FABMS (Xe; matrix, glycerol): *m/z* (relative intensity) 743 ($(M+1)^+$, 32).

2.1.3. 1-Eicosyl-2-phytanyl-sn-glycerol

1-Eicosyl-2-phytanyl-3-benzyl-*sn*-glycerol (1.6 g, 2.3 mmol) was added to a suspension of 5% palladium/carbon (0.5 g) in ethanol (30 ml) containing concentrated hydrochloride (3 drops), and the mixture was stirred magnetically under atmospheric pressure of hydrogen at room temperature for 17 h. The catalyst was removed by filtra-



1: (S)-DPhyPC and (RS)-DPhyPC

2: EPhyPC

3: HPhyPC

Fig. 1. Structures of the alkylphytanylglycerophosphocholines studied.

tion, and the solution was concentrated and applied to a silica gel column. Elution using hexane/ethyl acetate (8:1, v/v) gave a title compound as an oily substance, which showed a homogeneous spot in TLC; 0.5 g (34%); R_f, 0.56 (hexane/ethyl acetate (4:1, v/v)); $[\alpha]_D^{20}$ −3.6° (c 10, chloroform); ^{1}H-NMR (C^2HCl_3): δ 0.85 (m, 18H, $6CH_3$), 0.99–1.67 (complex m, 61H, $(CH_2)_{18}Me$ and $CH2CH_2(Me)CH[(CH_2)_3(Me)CH]_3Me$) and 3.36–3.70 (complex m, 9H, OCH and $4CH_2O$); FABMS (Xe; matrix, glycerol): m/z (relative intensity) 653 ($(M+1)^+$, 8).

2.1.4. 1-Eicosyl-2-phytanyl-sn-glycero-3-phosphocholine (EPhyPC)

A mixture of 1-eicosyl-2-phytanyl-*sn*-glycerol (0.48 g, 0.77 mmol), which was dried by azeotropic removal of a trace of water with benzene, and 2-bromoethylphosphonic dichloride (1 ml) in a sealed round bottom-flask was heated at 120°C (oil bath temperature) with stirring magnetically for 40 min. The cooled reaction mixture was agitated with water (ca. 20 ml) overnight. The resulting semi-solid substance was collected by decantation, washed several times with water and applied to a silica gel column. Elution with a mixture of chloroform and methanol (2:1, v/v) afforded 1-eicosyl-2-phytanyl-*sn*-glycero-3-(2-bromoethyl)phosphate as a crystalline compound, which was positive to both the Dittmer–Lester reagent and Rhodamine 6G sprays; 0.41 g (64%); m.p. 181–184°C; R_f 0.53 (chloroform/methanol (2:1, v/v)); $[\alpha]_D^{20}$ +0.6° (c 8, chloroform/methanol (2:1, v/v)). Next, the phosphate (0.41 g, 0.49 mmol) and a 2.4 M dimethylformamide solution of trimethylamine (about 5 ml) was heated at 50°C for 50 h, concentrated and applied to a silica gel column. Elution with chloroform/methanol/concentrated ammonia (65:35:5, v/v/v) furnished the crude EPhyPC in the fraction which was positive to both 0.25 mM Rhodamine 6G and the Dittmer–Lester reagent sprayings. The compound was further purified to a crystalline colorless substance by the use of Sephadex LH-20 gel column chromatography using chloroform/methanol (2:1, v/v); 0.32 g (79%); m.p. 175–178°C; R_f 0.54 (chloroform/methanol/concentrated ammonia (65:35:1, v/v/v)); $[\alpha]_D^{20}$ +0.8° (c 8, chloroform/methanol (2:1, v/v)); IR (KBr): 3400 (broad), 2930 (s), 1465 (s), 1230 (s), 1095 (s), 970 (m) cm^{-1}; ^{1}H-NMR ($C^2HCl_3/C^2H_3O^2H$ (2:1, v/v)): δ 0.79–0.91 (m, 18H, $6CH_3$), 0.99–1.72 (complex m, 61H, $(CH_2)_{18}Me$ and $CH_2CH_2(Me)CH[(CH_2)_3(Me)CH]_3Me$), 3.20 (s, 9H, $N^+(CH_3)_3$), 3.38–3.52 (m, 2H), 3.52–3.69 (m, 6H), 3.88 (t, 2H) and ca. 4.3 (br, 2H, NCH_2); FABMS (Xe; matrix, glycerol): m/z (relative intensity) 818 ($(M+1)^+$, 90).

2.1.5. 1-Hexadecyl-2-phytanyl-sn-glycero-3-phosphocholine (HPhyPC)

The lipid was derived from 1-hexadecyl-3-benzyl-*sn*-glycerol by the synthetic reactions similar to those mentioned above. The lipid was purified by a combination of silica gel- and Sephadex LH-20 gel column chromatography; overall yield, about 11%; m.p. 181–186°C; R_f 0.50 (chloroform/methanol/concentrated ammonia (65:35:1, v/v/v)); $[\alpha]_D^{20}$ +1.1° (c 10, chloroform/methanol (2:1, v/v)); IR (KBr): 3400 (broad), 2930 (s), 1465 (s), 1235 (s), 1095 (s), 970 (m) cm^{-1}; ^{1}H-NMR ($C^2HCl_3/C^2H_3O^2H$ (2:1, v/v)): δ 0.80–0.91 (m, 18H, $6CH_3$), 1.17–1.40 (m, 48H), 1.46–1.67 (m, 4H), 3.22 (s,

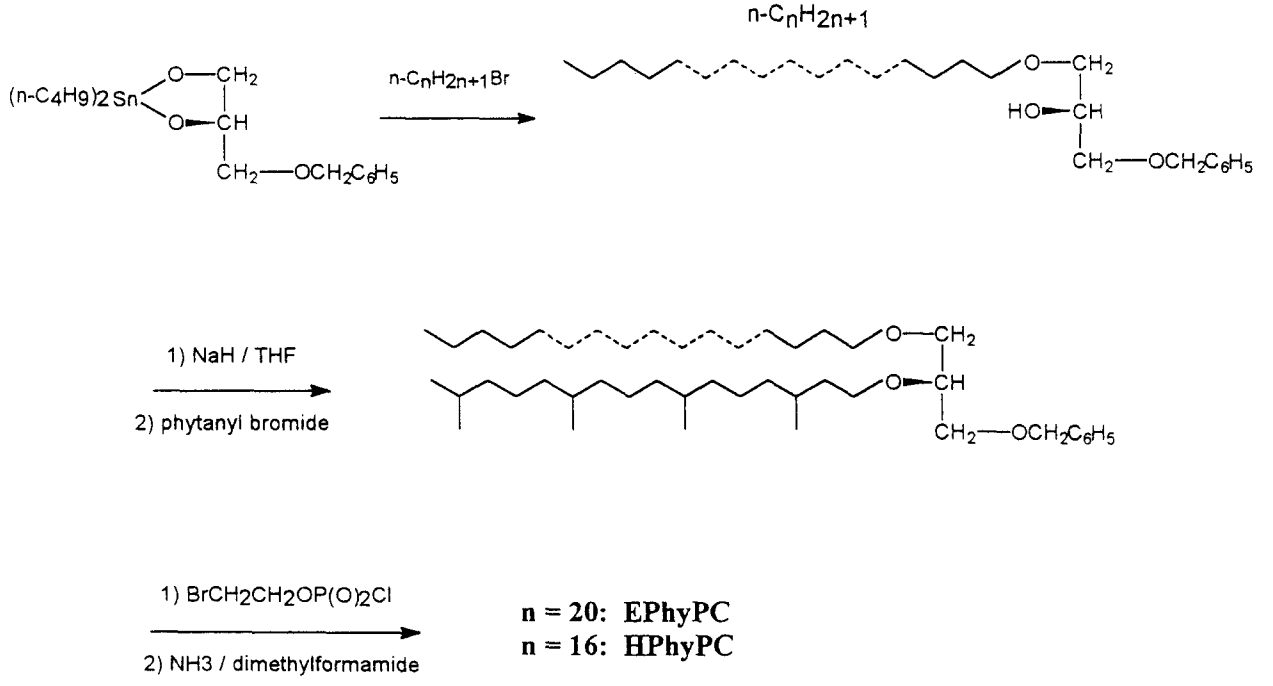


Fig. 2. Synthetic reaction pathways of EPhyPC and HPhyPC.

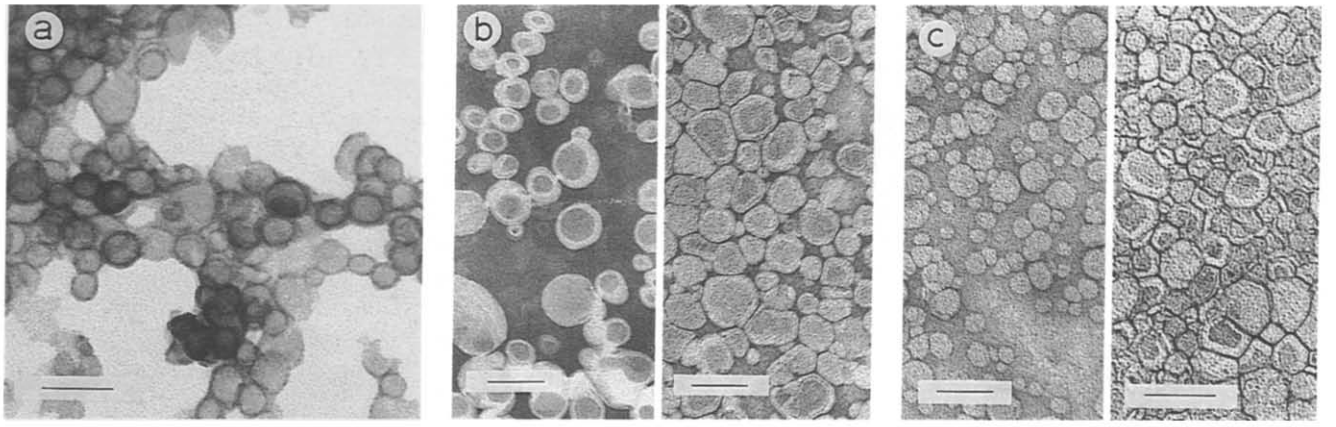


Fig. 3. Transmission electron micrographs. Bar = 1000 Å. Negative staining with uranyl acetate. (a) (*S*)-DPhyPC; (b) EPhyPC; (c) HPhyPC.

9H, $N^+(CH_3)_3$), 3.41–3.51 (m, 2H), 3.51–3.67 (complex m, 4H), 3.88 (t, 2H, CH_2CH_2N) and ca. 4.3 (br, 2H, NCH_2); FABMS (Xe; matrix, glycerol): m/z (relative intensity) 762 ($(M+1)^+$, 27).

2.2. Sonication of lipids in aqueous media

Lipids (EPhyPC, HPhyPC, etc., 2 mg) in distilled water (2 ml) was sonicated at 250 W per cm^2 of a titanium tip-cross sectional area and at 50–55°C for 20 min, centrifuged at about 2000 × *g* for 10 min. After annealing at 30°C for 1 h, the resulting aqueous solution was applied to a Sephadex G-25 gel column (20 mm × 30 cm) equilibrated with distilled water and eluted with the same media. The liposomes moved with the void volume (from 30 to 34 ml = about 4 ml). The clear suspension was subjected to light scattering measurements and transmission electron microscopic observation using 1.5 wt% aqueous uranyl acetate (pH 7) as a staining agent [14]. Typical micrographs and diameter-distribution of the assemblies are displayed in Figs. 3 and 4, respectively.

2.3. Phase transition measurements

Lipids (8 mg) in 25% aqueous ethylene glycol (0.5 ml) was vortexed for a few minutes and sonicated at 50°C for 20 min. The resulting aqueous solution weighed accurately in a range of 20–25 mg was placed in a aluminum pan, then the endothermic T_m and ΔH values were recorded by means of the highly sensitive calorimeter upon scanning temperature from −20 to 70°C at a rate of 1 K/min; experimental error, T_m ±0.5°C, ΔH ±0.5 kcal/mol. Results are listed in Table 1.

2.4. Surface pressure and surface area isotherm

The film balance system consisted of a Teflon-coated trough of 506 mm (length) × 150 mm (width), a Teflon-barrier and a microprocessor (NEC, PC-9801) which controlled the bar movement to collect the pressure vs. area data. Temperature (5, 10 and 20°C) of the subphase (water) was thermostated with circulating water within the trough. A benzene/ethanol solution (9:1, v/v, 30–45 μl) of lipid (2–3 mg/ml) was placed on the surface of the subphase, then the barrier was swept to compress the film at speed of 1 point/s or 60 mm^2/s. The pressure (π) was calibrated

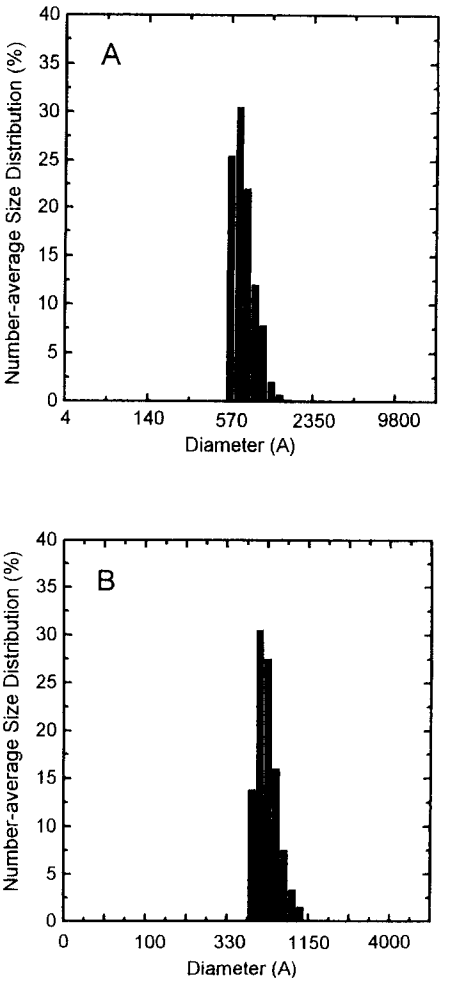


Fig. 4. Diameter distribution of aqueous liposome-suspesnion by light scattering measurements. (A) EPhyPC; (B) HPhyPC.

Table 1
Phase transitions of liposomal membranes

Lipid	T_m (°C)	Temperature of transition (°C)	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} deg^{-1})	ΔS per CH_2 unit (cal mol^{-1} deg^{-1})
DPPC	41.4	38 to 44	8.7	27	0.90
DHPC [a]	45.5	41 to 50	9.2	29	0.91
HPhyPC	−10.8	−17 to −7	3.7	14	0.43
EPhyPC	−11.1	−17 to −7	5.7	22	0.61
(*S*)-DPhyPC [b]	< −30	not obtainable [c]			

The data were obtained from heating curves in differential scanning calorimeter.
[a] From Ref. [23]. [b] From Ref. [13]. [c] No phase transition was observed from −30 to 70°C.

Table 2
Limiting occupied areas and maximum collapsing pressure of various Langmuir membranes

Lipid	Limiting area (Å^2) per					Collapsing pressure (mN/m)
	lipid molecule			phytanyl group	n-alkyl group	
Temp.	5°C	10°C	20°C	5–25°C	5–25°C	5–20°C
DPPC	57	60	60	–	≈ 25	≈ 59
DHPC	50	50	51–53	–	≈ 25	62–64
HPhyPC	80	80	81	55 [a]	26 [a]	50–51
EPhyPC	92	n.m. [b]	93	55 [a]	38 [a]	47–50
(*S*)-DPhyPC	104	106	116	52–58	–	47–50

[a] The limiting occupied area per alkyl chain was estimated by subtracting 55 Å^2 (of a phytanyl chain) from the observed occupied area per molecule; for instance, 38 Å^2 = 93–55 Å^2.
[b] Not measured.

by means of stearic acid-monolayer. The results are summarized in Table 2.

2.5. Leakage study of CF from liposomal assemblies

A mixture of lipid (5.0 mg) and 0.2 M aqueous solution of CF (0.6 ml) was sonicated as mentioned above, centrifuged, then the resulting homogeneous supernatant was applied to a gel column (2 cm × 25 cm) [Sephadex G-50 (medium)] equilibrated with 0.05 M Tris-HCl (pH 7.8) buffer and developed with the same solvent. The assembles bearing CF probes in the inner domain were eluted first (1.5–3 ml) and separated from the latter band containing free probes. The aqueous suspension (30 μl) of the assemblies was then diluted with the Tris-HCl buffer (3.0 ml), and the fluorescence intensity (I_t) at 520 nm with excitation at 490 nm (band width: excitation, 10 nm; emission 5 nm) was measured at a suitable temperature. The I_t increased proportionally with the amount of the probe released into the outer aqueous phase. After the measurement, the sample solution was mixed with a 10 vol% aqueous Triton X-100 solution (5 μl), and the maximum intensity (I^*) resulting from instantaneous rupture of the membranes was measured. The extent (%) of leakage is given by the $100(I_t/I^*)$ and plotted against time as

Table 3
Cumulative leakage extent (%) of CF from various liposomes as a function of incubation period (Inc.) at ambient temperature; solvent, 0.05 M Tris-HCl (pH 7.8)

	Cumulative leakage extent (%) of CF								
Inc. (day)	1	5	7	9	12	13	21	28	35
DPPC	15	65	80	88	95	95	–	–	–
(*S*)-DPhyPC	3	6	7	9	10	11.5	16	19	22
EPhyPC	2	3	4	4.5	5.5	5	8	10	12

Table 4
Cumulative leakage extent (%) of Cl^- ions from various liposomes as a function of incubation time (Inc.) at 20°C and at 40°C

	Cumulative leakage extent (%) of Cl^- ions								
Inc. (min)	2	4	6	8	10	12	14	16	18
20°C									
DPPC	35	45	49	50	53	54	55	56	57
HPhyPC	6	8	10	11	13	14	15	16	17
(*S*)-DPhyPC	2	5	7	8	10	11	11	12	13
EPhyPC	4.5	6	8	9.5	10	11	12	12	13
40°C									
DPPC	55	80	89	93	95	–	–	–	–
HPhyPC	–	8	13	17	23	28	35	38	42
(*S*)-DPhyPC	4	7	10	13	16	20	27	27	30
EPhyPC	–	7	11	14	18	20	24	28	32

The aqueous interior and exterior of the liposomes were 1 M NaCl and 1 M $NaNO_3$, respectively.

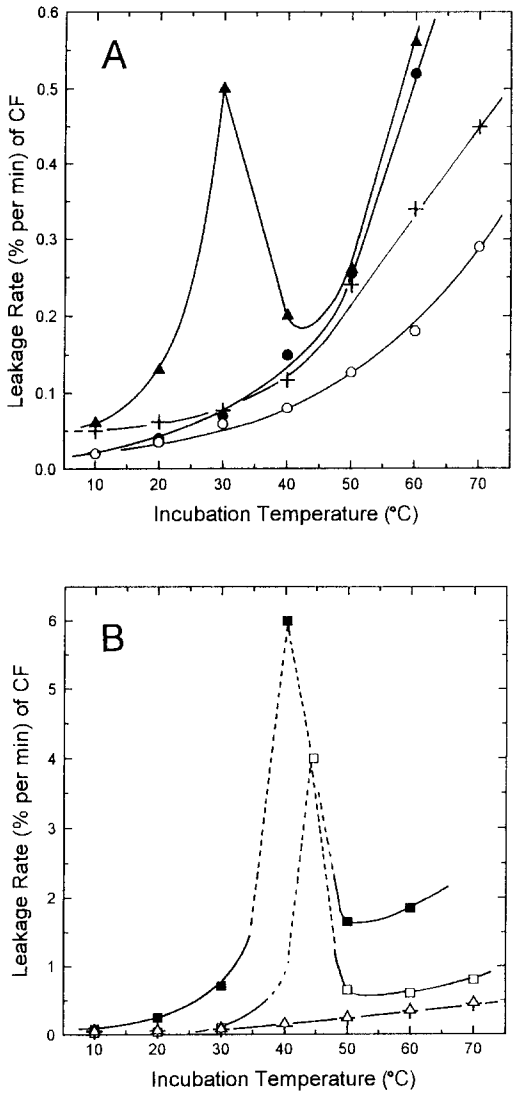


Fig. 5. Initial rate of leakage (% per min) of CF from the liposomes as a function of incubation temperature. Solvent, 0.05 M Tris-HCl (pH 7.8). Panel A (the ordinate is enlarged): EPhyPC (○), HPhyPC (●), (*S*)-DPhyPC (+) and a 1:1 molar mixture of HPhyPC and DPhyPC (▲). Panel B: DPPC (■), DHPC (□), (*S*)-DPhyPC (+) and (*RS*)-DPhyPC (△).

shown typically in Fig. 5 and Table 3. The observed intensity was calibrated to the value at 25°C. A separate experiment showed that the quenching of CF was eliminated by diluting the dye below about 10 mM.

2.6. Leakage study of Cl^- ions from liposomal assemblies.

Lipid (EPhyPC, etc. 15 mg) was sonicated in 1.0 M sodium chloride (3 ml) at 50°C for 25 min. The suspension was centrifuged to give the supernatant, which was then applied to a gel column (Sephadex G-50 (fine)) equilibrated with 1.0 M sodium nitrate. Elution with 1.0 M sodium nitrate gave the liposomes containing isosmotic aqueous sodium chloride in the liposome interior (= 1.8 osmol/kg). The suspension in a glass beaker was then kept at suitable temperature and the leakage of Cl^- ions was measured by means of a chloride ion-specific electrode. Representative results are shown in Table 4.

3. Results and discussion

1-Eicosyl- and 1-hexadecyl-3-benzyl-*sn*-glycerols, the key intermediates of nonnatural HPhyPC and EPhyPC, were produced readily by a regioselective etherification of 1,2-(dibutyl)stannylene-3-benzyl-*sn*-glycerol with the corresponding alkyl bromide (Fig. 2) [14]. The straight alkyl chains are equal to or longer but less bulky than the phytanyl group. We chose a phosphocholine group as a polar residue because many lipids bearing the bulky hydrophilic moiety tend to form readily liposomal membranes [20,21].

Now, DPhyPCs (**1**), EPhyPC (**2**) and HPhyPC (**3**), upon sonication in water at 20–50°C followed by gel-column chromatography of the resulting suspension, furnished the well-defined liposomes, most of which were unilamellar in structure. The vesicles were 200–1000 Å in diameter according to an electron microscopic observation (Fig. 3a–c) and a light scattering-measurement (Fig. 4a,b). In comparison with the aqueous liposome suspension from HPhyPC, those from DPhyPCs and EPhyPC were stable to preserve the morphology for at least a month at ambient temperature. The main phase transition constants of various membranes, perhaps assignable to a gel-to-liquid crystalline phase transition, are listed in Table 1. As anticipated, the transition temperature (T_m) of the HPhyPC- and EPhyPC-membranes appeared somewhere between those of (*S*)-DPhyPC and the corresponding straight chain-lipids such as DHPC [1]. The ΔS/methylene unit, which may reflect the orderliness of the hydrocarbon chains at the phase transition, was increased in the following order: ((*S*)-DPhyPC) < HPhyPC < EPhyPC < DHPC ≈ DPPC; here, the phytanyl chain was counted as a 16-methylenes unit. By the way, both EPhyPC and HPhyPC gave monolayers on a water/air interface, allowing their n-alkyl and phytanyl chains to occupy 30–40 and 50–60 $Å^2$/chain, respectively, at maximum compression and 5–20°C (Table 2). By contrast, the alkyl chains of DHPC- and DPPC-monolayers covered an area of only 20–25 $Å^2$/chain under the same conditions. Although a monolayer in a water/air interface may not be related directly to a lipid bilayer in water, the DSC studies and the surface area/surface pressure isotherm studies suggest that the n-alkyl chains in the EPhyPC- and HPhyPC-liposomal

[1] A simple 1:1 molar mixture of DPhyPC and DHPC gave liposomes which underwent a phase transition from −15 to −7°C centering at −11.8°C (= T_m).

membranes were oriented rather randomly even at the low temperature.

Next, we examined permeability of liposomal membranes. As reported already, the liposomes made of (*S*)-DPhyPC and other archaebacterial model lipids gave the vesicles which could retain efficiently water-soluble substance over a wide temperature range [11–13]. However, even at *low* temperature (10–20°C) these artificial membranes were not completely leak-free; for instance, it was found in the (*S*)-DPhyPC-liposome system that the trapped solutes such as CF escaped from an aqueous interior to an outer aqueous phase in a rate of about 0.05% as shown in Fig. 5a, line +; c.f., permeability of the (*RS*)-DPhyPC-liposomes was essentially similar to that from (*S*)-DPhyPC. The leakage rate was small, but the solute accumulated in an outer phase, amounting, for instance, about 20% of an originally trapped quantity within a few weeks; see Table 3. In this respect, it would be remarkable that the ordinal lipids having straight hydrocarbon chains provided the membranes of high barrier property at a low temperature (Fig. 5b, lines ■ and □). We have assumed that (a) the DPhyPC-membrane ($T_m < -20$°C) was too fluid to function as a tight barrier, whereas (b) the DPPC-, DHPC- and other PC-membranes were in a rigid gel phase at the low temperature range to render their membranes impermeable. It, hence, would be worth noting that the vesicles from the chimerical lipids, particularly EPhyPC, could keep the dye well in the interior from low to high temperature and even exceeded the (*S*)-DPhyPC-liposomes as demonstrated in Fig. 5a and Table 3. By contrast, the liposomes made of a simple 1:1 molar mixture of (*S*)-DPhyPC and DHPC were leaky as displayed in Fig. 5a, line ▲. The EPhyPC-vesicle functioned also as a good container for NaCl whereas the inorganic salt escaped readily from the DPPC- (and also DHPC-) liposomes at temperatures $T \gtrsim T_m$ and at high salt concentration (1–3 M $NaNO_3$) as demonstrated in Table 4.

Although the high barrier property, particularly of the EPhyPC-liposomal membrane, has not been explained well, the result might be ascribed partly to (i) a steric hindrance in the bulky polyisopranyl layer against a free passage of the substances and (ii) to an increased rigidity (the large T_m and ΔH in comparison with those of the DPhyPC membrane) by the presence of the straight alkyl chains. Such as steric effect may also lead to a restricted bond-rotation and prevent the chain to form transient kinks, along which the permeants such as organics and metal ions have been considered to travel [22].

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