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Archaebacterial lipid models: formation of stable vesicles from single isoprenoid chain-amphiphiles

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Abstract

Surfactants, $PhyN^+(Me)_3$ (OH^-/Br^-), $PhyPO_4H^-$ (H^+/Na^+), $PhySO_4^-$ (H^+/Na^+) and $Phy(CO_2^-)$ (H^+/Na^+), were prepared; Phy is a (3*RS*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl group; phytanic acid or its sodium salt is expressed by $Phy(CO_2^-)$ (H^+/Na^+). Equimolecular mixtures of the cationic surfactant and the anionic ones in aqueous media, upon sonication, gave rise to stable suspensions of multilamellar vesicles (MLVs). Unlike the molecular assemblies from cationic and anionic pairs of straight chain-surfactants, the MLVs could trap ionic and nonionic compounds such as 5(6)-carboxyfluorescein and glucose with the captured volume of 0.6–1.6 l/mol in water. The vesicles were tolerant to about 25 mM aqueous NaCl. The packing mode of the surfactants in the membranes was discussed in conjunction with the thickness (about 60 Å) and the zeta-potential which was as large as 30–45 mV in water at 20°C.

Key words: Surfactant; Liposome; Archaebacterium; NMR, ^1H-; Fast atom bombardment mass spectrometry; Membrane morphology

1. Introduction

Archaebacteria proliferate in extreme habitats such as hot springs, salt lakes and acidic aqueous spots. At a molecular level, one of the most distinguished features appears in their lipids which always bear saturated polyisoprenoid chains [1–4]. We have shown that the bacterial lipids and model compounds such as 1,2-di-phytanyl-*sn*-glycero-3-phosphocholine (Fig. 1, **1**; the phytanyl = (3*RS*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl) formed highly stable liposomal membranes [5–7]. Chan and co-workers reported that a total lipid extract from *Halobacterium cutirubrum* formed the liposomes (MLV) which behaved as ideal osmometers in KCl or NaCl solutions [8]. The properties originated most likely from the isoprenoid-chains.

On the other hand, various pairs of cationic and anionic surfactants such as cetyltrimethylammonium hydroxide/palmitic acid ($C_{16}H_{33}NMe_3^+/C_{15}H_{31}CO_2^-$) have been reported to give vesicular assemblies [9,10]. We have also observed the large spherical assemblies as displayed in transmission electron microscopy (TEM) (Fig. 2a), but found that the spheres could not trap materials such as glucose and CF at all. The assemblies were decomposed even in 0.5 mM sodium chloride. In this paper we wish to describe a formation of stable multilamellar liposomes from 1:1 molar mixtures (Fig. 1, **2**) of the cationic and anionic surfactants bearing an isoprenoid chain. The surfactants were designed in the hope that they, like archaebacterial lipids, may form stable molecular assemblies (Fig. 3).

2. Materials and methods

Water was ion-exchanged, distilled twice and filtered through a Millipore Milli-Q Labo equipped with

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Abbreviations: CF, 5(6)-carboxyfluorescein; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (palmitoyl = $C_{15}H_{31}CO$-); DSC, differential scanning calorimetry; FABMS, fast atom bombardment mass spectrum; IR, infrared; MLV, multilamellar vesicle; SUV, small unilamellar vesicle; TEM, transmission electron microscopy; TLC, thin layer chromatography; THF, tetrahydrofuran; T_m, the temperature at midpoint in gel-to-liquid crystalline phase transition.

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a 0.22 μm filter unit. Phytanol (**4**) [(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethyl-1-hexadecanol)] and phytanyl bromide [(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethyl-1-hexadecyl bromide] were prepared previously from commercially available phytol (**3**) (Fig. 3) [11]. Thin-layer, column and gel-permeation chromatographies were carried out on a silica gel-precoated sheet (Merck Art. 5735), silica gel (Merck 7734, 70-230 mesh) and modified dextran (Sephadex G-50 and LH-20), respectively. Unless specified, the following solvents systems were used in the chromatographies: system code (solvent, volume ratio): A (chloroform/methanol, 2:1); B (chloroform/methanol/water, 65:25:4). Compound spots in TLC were visualized under ultraviolet light after spraying with 0.25 mM aqueous Rhodamine 6G (for the long hydrocarbon chains) and the Dittmer-Lester reagent (for the phosphoric acid ester) [12]. ^{1}H-NMR spectra were recorded on a Jeol GX-400 spectrometer using a dilute solution in 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 or a mixture of glycerol on a standard FAB target, then subjected to a beam of xenon atoms produced at 8 kV and 2 mA. Ultrasonication was preformed by the use of a probe-type ultrasonic disintegrator (Ohtake Works Co., model 5201). Phase transition was studied by means of a Rigaku 8240 and a Microcal MC-2 scanning calorimeters. Other instruments used were a Shimazu ST-1 surface tension balance, a Hitachi H-7000 transmission electron microscope, a Shimazu RF-502A spectrofluorometer, and a Ohtsuka DLS-700 light scattering spectrometer which was equipped with an electrophoretic mobility cell and controlled by a microprocessor, NEC-9801.

2.1. Preparation of isoprenoid surfactants

2.1.1. (3RS,7R,11R)-Phytanyl trimethylammonium bromide (PhyN$^+$Me$_3$Br$^-$)

A mixture of the phytanyl bromide (4.8 g, 13.3 mmol), aqueous trimethylamine (30 wt%, 70 ml), chloroform (30 ml), 2-propanol (50 ml) and acetonitrile (50 ml) was stirred at ambient temperature for 3 days. The reaction mixture was concentrated to give the residue which was applied to a silica gel column. Elution with a mixture of chloroform, methanol and water (65:25:4, by vol.) gave the colorless oil, which was passed subsequently through Sephadex LH-20 column using a solvent A to exhibit a homogeneous spot of PhyN$^+$Me$_3$Br$^-$ in TLC; viscous oil after freeze-drying from benzene/methanol (5:1 v/v); 4.7 g (84%); Rf 0.56 (solvent B); IR (neat): 2940 (s), 1460 (m), 1380 (m) and 970 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ0.838–0.887 (complex m, 15 H, 5 CH_3), 3.47 [s, 9 H, $N(CH_3)_3$]; FABMS (positive ion-detection mode; matrix, glycerol): *m/z* 340 [*M*(= trimethylphytanylammonium ion); relative intensity, 100%]. Analysis of Br^--ions [13] for $C_{23}H_{50}H_1Br_1$: calculated: 19.00; found: 18.72.

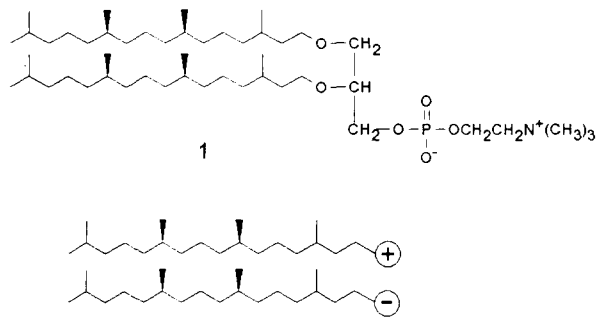

Fig. 1.

2.1.2. (3RS,7R,11R)-Phytanyltrimethylammonium hydroxide (PhyN$^+$Me$_3$OH$^-$)

PhyN$^+$Me$_3$Br$^-$ (0.20 g, 0.48 mmol) dissolved in a mixture of methanol and water (9:1, v/v) (10 ml) was stirred with Ag_2O (0.20 g, 0.87 mmol) under nitrogen gas untill the supernatant (acidified.by nitric acid) did not give precipitates of AgBr upon addition of aqueous silver nitrate. Silver bromide was removed by means of a glass filter to give a 25 mM aqueous methanol solution (about 18 ml) of PhyN$^+$Me$_3$OH$^-$; yield, 94%; the surfactant concentration was measured by titration with 0.1 M aqueous hydrochloric acid. The NMR spectrum was essentially identical with that of PhyN$^+$Me$_3$Br$^-$.

2.1.3. (3RS,7R,11R)-Phytanic acid [(3RS,7R,11R)-3,7,11,15-tetramethylhexadecanoic acid] [Phy(CO_2H)]

A reaction of the phytanol (3.82 g, 12.8 mmol) with pyridine–SO_3complex (16 g, 0.10 mol) produced 1-phytanal [(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethyl-1-hexadecanal] quantitatively. The aldehyde was stirred at ambient temperature for 1 h with 2-methyl-2-butene (3.6 g, 51 mmol), $NaH_2PO_4 \cdot H_2O$ (1.9 g, 12 mmol), sodium chlorate (3.6 g, 40 mmol), water (22 ml) and t-butanol (84 ml). The resulting phytanic acid was purified by a combination of a silica gel column using chloroform and a Sephadex LH-20 column using solvent A and freeze-dryed from benzene/methanol (5:1, v/v); colorless oil, 1.82 g (48%); a homogeneous spot at Rf 0.80 (chloroform); ^{1}H-NMR ($CDCl_3$): δ 0.836–0.886 (complex m, 15H, 5CH_3), 2.213 (complex d, 2H, CH_2CO); FABMS (negative ion-detection mode; matrix, glycerol): *m/z* 311 (*M* − 1; relative intensity, 100%); IR (neat) 2940 (s), about 2550–2750 (broad, COOH), 1705 (s, carbonyl), 1460 (m), 1300 (m) cm^{-1}.

The IR and NMR spectra were agreed with those reported in the literature [14].

2.1.4. (3RS,7R,11R)-Phytanyl phosphate ($PhyPO_4H_1^-Na^+$)

The phytanol (1.1 g, 3.7 mmol), which was dried by azeotropic distillation of benzene, was dissolved in an hydrous tetrahydrofuran (30 ml), cooled to about 0°C, and phosphoryl chloride (7 ml) was injected slowly to the stirred alchohol solution. After addition, the reaction mixture was stirred at ambient temperature overnight. The resulting solution was cooled and stirred with water overnight, then adjusted to pH 7 by 1 M aqueous NaOH and fractionated between chloroform and water. The concentrated organic layer was applied to Sephadex LH-20 column (2.5 cm × 60 cm). Elution with the solvent A furnished $PhyPO_4H_1^-Na^+$, which was obtained as viscous oil after freeze-drying from

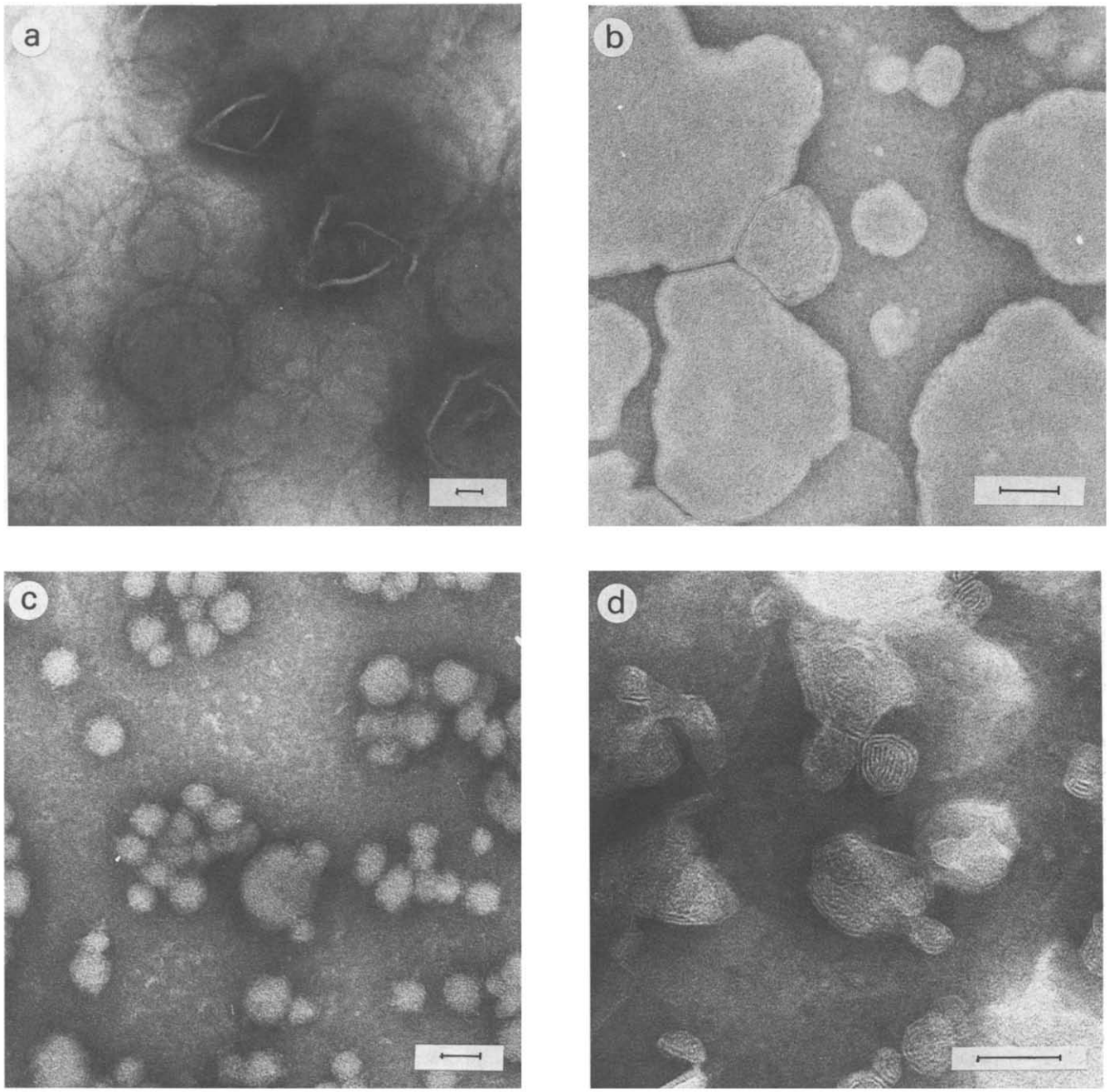

Fig. 2. Transmission electron micrographs; negative staining with phosphotungstic acid/sodium hydroxide (pH 7). The bar is 1000 Å. (a) $C_{16}H_{33}N^+Me_3/C_{15}H_{31}CO_2^-$, (b) $PhyN^+Me_3/Phy(CO_2^-)$, (c) $PhyN^+Me_3/PhyPO_4H^-$, (d) $PhyN^+Me_3/PhySO_4^-$.

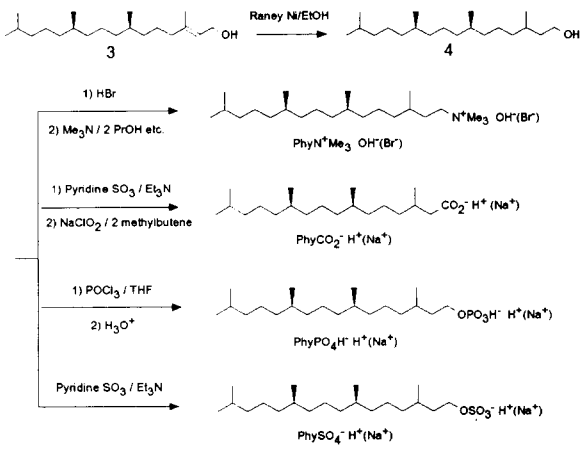

Fig. 3. Synthesis of isoprenoid cationic and anionic surfactants.

benzene-methanol (5:1, v/v); 0.65 g (43%); a homogeneous spot at Rf 0.30 (solvent B); IR (neat): 2940 (s), 1170 (s, P-O), 1075 (vs, P-O), 940 (s, P-O); ^{1}H-NMR ($CDCl_3$): δ0.835–0.888 (complex m, 15 H, $5CH_3$), 3.561 (broad, 2H, CH_2O); FABMS (positive ion-detection mode; matrix, glycerol): *m/z* 401 ($PhyPO_4H^-Na^+ + H^+$; relative intensity, 55%) and 423 ($PhyPO_4H^-Na_2^+$; 45%).

2.1.5. (3RS,7R,11R)-Phytanyl sulfate ($PhySO_4^-Na^+$)

The phytanyl alcohol (3.56 g, 11.9 mmol), which was dried by azeotropic distillation of benzene, was dissolved in an hydrous tetrahydrofuran (100 ml), cooled to about 0°C, then mixed with sulfur trioxide pyridine complex (5.6 g, 35.2 m mmol). The solution was warmed to ambient temperature and stirred magnetically overnight. The resulting reaction mixture was agitated with water, neutralized with 1 M aqueous NaOH, and the concentrated solution was applied to silica gel column. Elution with solvent B furnished $PhySO_4^-Na^+$, which was purified subsequently by means of Sephadex LH-20 column using solvent A to give colorless powder; freeze-dryed from benzene/methanol (5:1, v/v); 3.7 g (78%); a homogeneous spot at Rf 0.58 (solvent B); m.p. 167–170°C; IR (neat): 2940 (s), 1245 and 1220 (vs, S-O), 980 (s, S-O); ^{1}H-NMR ($CDCl_3$): δ0.847–0.881 (complex m, 15 H, 5 CH_3), 4.099 (t, 2 H, CH_2OS, J = 3.0 Hz); FABMS (negative ion-detection mode; matrix, glycerol): *m/z* 377 ($C_{20}H_{41}SO_4^-$ ion; relative intensity, 100%).

2.1.6. $PhyPO_4H_2$ and $PhySO_4H$

The Na^+-form of the phytanyl surfactants above prepared were ion-exchanged by means of the column packed with Dowex 50 WX (H^+-form). The resulting acid-forms were passed through a Sephadex LH-20 gel column using solvent B. All of them were obtained as viscous oil after freeze-drying from benzene-methanol (5:1, v/v) to show the corresponding homogeneous spots in TLC. Their FABMS, IR and ^{1}H-NMR spectra were essentially similar to those of the Na^+-forms.

2.1.7. CMC measurements

The methodology was based on the Whilhelmy's method. About 10^{-3} M aqueous solutions of the phytanyl surfactants and the surfactant-pairs were prepared as stock solutions. Each of them was diluted with water to various apparent concentrations and sonicated at 50°C for 25 min, and after the weight was readjusted by addition of water, surface tension was measured at 20 ± 2°C by means of the balance equipped with a flat glass tip. The CMC was regarded as the concentration which showed a well-defined refraction in the plots of the surface tension vs. concentration; experimental error: ±3% and ±10% for the surfactants and their pairs, respectively.

2.1.8. Preparation of molecular assemblies

Each of the surfactants was dissolved in chloroform to obtain their 10 mM stock solutions. The stock solutions were combined to afford various 1:1 molar mixtures of cationic and anionic surfactants. The solutions were concentrated, kept in a desiccator with phosphorus pentaoxide (as desiccant) overnight under reduced pressure, and sonicated in water or a buffer (3 ml/about 5.0–6.0 mg of a surfactant pair) at 35 W for 20 min. The aqueous medium, if necessary, contained 0.2 M glucose or 40 μM CF. The resulting clear suspension was centrifuged at 2000 × *g* for 15 min to give the supernatant, which was used in the following studies.

2.1.9. Transmission electron microscopy

A half drop of the sonicated surfactant-suspension prepared above was laid on the collodion film (on a copper grid) which was coated with a thin carbon (about 10 nm thickness). A half drop of 1% (w/v) aqueous phosphotungstic acid/sodium hydroxide (pH 7) or aqueous uranium acetate was then added to the suspension on the grid, and staining was allowed to proceed for 2–5 min at room temperature. The excess liquid on the grid was removed by means of the tip of an absorbing paper to give the specimen which was mounted to the electron microscope to observe the image of the molecular assemblies at a magnification of $1–2 \times 10^4$. The picture was usually enlarged by a factor of 5–10.

2.1.10. Light scattering

The sonicated surfactant-suspensions above obtained were filtered through a cellulose acetate membrane (Corning #21033-13; pore size, 0.45 μm), and scattering intensity and electrophoretic mobility of particles were measured at an angle of 90° and at 25 ±

0.5°C using DLS- and ELS-standard cells, respectively. The instrument had been programmed to calculate and print out automatically a number-average distribution of particle size as well as ζ-potential from the scattering and mobility data. The results are listed in Table 2.

2.1.11. Entrapped volume

A mixture of the surfactant mixture (10–20 mg) and an aqueous 0.3 M glucose or 40 μM CF (2 ml) was sonicated at 45°C and 35 W for 30 min; if necessary, the sonication was conducted in the presence of 1–50 mM NaCl. The resulting suspension was applied to a Sephadex G-50 column (2 cm × 50 cm) equilibrated with an isoosmotic aqueous ethylene glycol. The eluent was fractionated to collect 2.5 ml in each glass tube. A concentration of glucose in the fractions was determined by mixing with a 10 vol% aqueous Triton X-100 solution (10 μl) (to rupture the assemblies) and using the glucose analysis kit (Wako Chemicals, product No. 271–3041), which consisted of glucose peroxidase, peroxidase, 4-aminoantipyrine and phenol in 30 mM (pH 7.4) phosphate buffer. CF was quantified from the fluorescence intensity at 520 nm with excitation at 470 nm (band width: 5 nm for both modes) [5]. The concentrations of $PhyPO_4H^-$ and $PhyN^+Me_3$ ions were estimated by means of the phosphorus- and modified nitrogen-microanalyses by Bartlett [15] and Sloane–Stanley [15], respectively. A molar ratio, cationic surfactant/anionic surfactant, in the assemblies was determined conveniently from an area-ratio of signals of the $N^+(CH_3)_3$ moiety of the $PhyN^+Me_3$ moiety and the respective α-CH_2 signals of the anionic surfactant ions in the ^{1}H-NMR spectra ($CDCl_3$) of the concentrated fractions (their the chemical shifts are described in section 2). As displayed for example in Fig. 4, a front band containing the assemblies with glucose in the aqueous interior was separated well from a latter band containing the free probes. Entrapped volume was calculated from the amounts of the entrapped probes (glucose or CF) and surfactants upon assuming that a concentration of the probes in the assemblies was the same as that of the original solution.

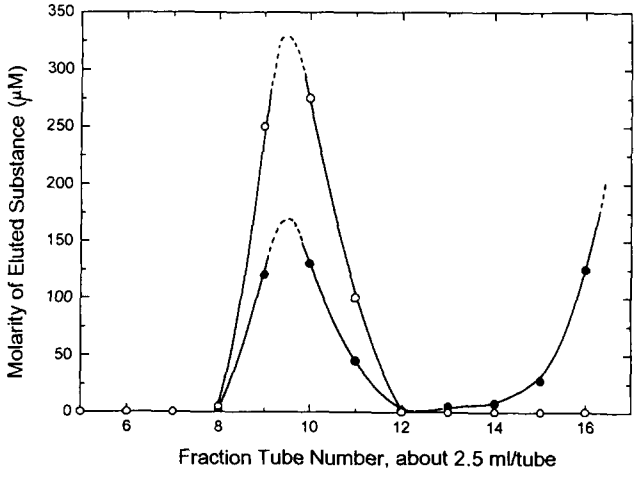

Fig. 4. The gel permeation chromatograph (Sephadex G-50; 2×30 cm) of the sonicated aqueous suspension of $PhyN^+(Me)_3/PhyCO_2^-$ in 0.3 M glucose. Each tube contained about 2.5 ml of the eluent. ○, the surfactant pair; ●, glucose.

Table 1
CMC values of various single chain-surfactants and their equimolar mixtures

Substance [a]	CMC at 20°C (mol/l)
$n\text{-}C_{16}H_{33}N^+Me_3Br^-$	$9.2\cdot10^{-4}$
$n\text{-}C_{16}H_{33}SO_4^-Na^+$	$5.2\cdot10^{-4}$
$n\text{-}C_{15}H_{31}CO_2^-Na^+$	$5.5\cdot10^{-4}$
$PhyN^+Me_3Br^-$	$1.4\cdot10^{-3}$
$Phy(CO_2^-)Na^+$	$1.6\cdot10^{-3}$
$PhyPO_4H^-Na^+$	$1.3\cdot10^{-3}$
$PhySO_4^-Na^+$	$3.0\cdot10^{-4}$
$PhyN^+Me_3/Phy(CO_2^-)$	$7.7\cdot10^{-6}$
$PhyN^+Me_3/PhyPO_4H^-$	$4.9\cdot10^{-6}$
$PhyN^+Me_3/PhySO_4^-$	$5.3\cdot10^{-6}$

[a] The phytanyl-surfactants are shown in Fig. 3.

3. Results

3.1. CMC values of the phytanyl surfactants

The amphiphiles were prepared easily in an overall yield (from phytanol or phytanyl bromide) of 70–95% via the reactions displayed in Fig. 3. Like many isoprenoid lipids, the phtanyl surfactants except $PhySO_3^-Na^+$ were viscous oil even at −20°C. They were readily dissolved in water, and the solution, after sonication, exhibited CMC values in an order of 10^{-3} M (Table 1). The phytanyl surfactants are similar in length to n-hexadecyl analogues, but the former had larger CMC values than the latter.

3.2. Morphology of molecular assemblies

An addition of an aqueous solution of a phytanyl-surfactant to an equimolar aqueous solution of an oppositely charged surfactant resulted in precipitating instantly the white mass. However, sonication of the homogeneous equimolar surfactant mixtures, $PhyN^+(Me)_3/Phy(CO_2^-)$, $PhyN^+(Me)_3/PhyPO_4H^-$ and $PhyN^+(Me)_3/PhySO_4^-$, gave rise to a clear suspension of MLVs (Fig. 2b–d) of about 500 ~ 600 Å in number-average diameter. The NMR spectra of the concentrated aqueous suspensions (solvent: deuterium oxide) revealed that the molecular assemblies were composed of 1:1 molar mixtures of the corresponding cationic and anionic amphiphiles. The membranes had 57–75 Å in

thickness as measured from the layer periodicity of the stacked layers in TEM. The diameter and thickness are summarized in Table 2.

3.3. Zeta (ζ)-potentials

The MLVs of the phytanyl surfactant-pairs and the molecular assemblies of $C_{16}N^+Me_3/C_{16}CO_2^-$ possessed as ζ-potential as large as + 30–40 mV in water at 20°C (Table 2). By contrast, the liposomal membranes, which were made of the lipids bearing an isoelectric or neutral interface such as 1 (Fig. 1) and egg yolk phosphatidylcholine, had +3–6 mV under the similar conditions.

3.4. DSC

High sensitivity scanning calorimeters did not exhibit any phase transition for the aqueous suspensions of the phytanyl surfactant pairs at temperature from −20 to 70°C. An absence of the phase transition has been reported in most liposomal membranes from archaebacterial lipids [16,17].

3.5. Entrapped volume of MLVs

The MLVs were eluted simultaneously with glucose or CF in the gel permeation chromatography (Fig. 4). Upon addition of Triton X-100 to the suspensions, the captured probes were released instantaneously from the vesicles into a bulk phase. The entrapped volumes (liter/mol) are listed in Table 3. $Phy(CO_2^-)/PhyN^+(Me)_3$ provided the largest volume, which was comparable with ordinary MLVs of glycerophospholipids such as **1** and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) [18].

By contrast, straight chain–surfactant pairs such as $C_{16}H_{33}N^+Me_3/C_{15}H_{31}CO_2^-$ and $C_{16}H_{33}N^+Me_3/C_{16}H_{33}SO_4^-$ could trap neither glucose nor CF. The morphology (Fig. 2a) was lost or became undefined at > about 0.5 mM ionic compounds such as NaCl, CF and L-ascorbic acid sodium salt.

Table 2
MLVs from various surfactant-pairs: number-average diameter, thickness of the membrane and zeta (ζ)-potential

Surfactant pair	Diameter [a] (Å)	thickness (Å)	ζ-potential [b] (mV)
n-$C_{16}H_{33}N^+Me_3$/n-$C_{16}H_{33}SO_4^-$	840	[d]	+25±3
n-$C_{16}H_{33}N^+Me_3$/n-$C_{15}H_{31}CO_2^-$	710 (30–10) [c]	[d]	+30
$PhyN^+Me_3/Phy(CO_2^-)$	560	61±5	+45
$PhyN^+Me_3/PhyPO_4H^-$	610	75±8	+30
$PhyN^+Me_3/PhySO_4^-$	560	57±5	+35
DPhyPC (Fig. 1, **1**)	560	63±3	+6

[a] The diameter at the highest population of the various sizes of MLVs.
[b] Measured in water at 20°C.
[c] From Ref. [9].
[d] Not determined.

Table 3
Entrapped volume in MLVs of surfactant-pairs at various NaCl-concentrations [a]

Surfactant-pair	Entrapped volume (l/mol)				
[NaCl] (mM):	0	1	10	25	50
n-$C_{16}H_{33}N^+Me_3$/n-$C_{16}H_{33}SO_4^-$	0	0	0	0	0
n-$C_{16}H_{33}N^+Me_3$/n-$C_{15}H_{31}(CO_2^-)$	0	0	0	0	0
$PhyN^+Me_3/PhyCO_2^-$	1.6	1.4	1.3	1.0	[b]
$PhyN^+Me_3/PhyPO_4H^-$	1.1	1.0	1.0	0.8	[b]
$PhyN^+Me_3/PhySO_4^-$	0.6	0.6	0.4	0.3	[b]

[a] Glucose was used as a probe for determination of the entrapped volume of MLVs; assay temperature, 20±3°C.
[b] Precipitate formation.

3.6. Effects of NaCl

The entrapped volume of the assemblies, which were prepared from surfactant pairs in a various NaCl-concentration (1–50 mM), are displayed in Table 3. The volume decreased with increasing the salt concentration. All assemblies lost their trapping capability in 50 mM NaCl. At higher concentration the surfactants were precipitated.

4. Discussion

Equimolecular mixtures of the cationic and anionic surfactants (Fig. 3; the OH^- and H^+ forms, respectively) gave the CMC values ($4.9–7.7 \times 10^{-6}$ M) which were much smaller than those ($0.3–1.6 \times 10^{-3}$ M) of the individual surfactants (Table 1). Upon sonicating in water, they formed the MLVs with the same 1:1 molar composition as the starting surfactants. Some of MLVs could trap glucose and CF as demonstrated typically in the gel permeation chromatography (Fig. 4). It would be noteworthy that (a) the entrapped volume (0.7–1.6 l/mol; Table 3) was as large as that of ordinary liposomes of glycero-phospholipids [18] and (b) the vesicles were tolerant to 25–50 mM aqueous NaCl (Table 3). By contrast, the assemblies (Fig. 2a) from the straight chain surfactants could not store neither ionic compounds nor neutral glucose. The assemblies were also decomposed even in 0.5 mM NaCl.

The membranes of the MLVs were consisted of the cationic and anionic surfactants with an apparent molar ratio of 1:1. The observed thickness (57 ~ 75 Å; Table 2) was approximately two-fold of the molecular length of the surfactants (about 10 Å of a polar head + 20 Å of a phytanyl chain). Ordinary bilayers of glyc-

erophospholipids such as DPPC are about 50–55 Å in thickness [19]. These suggest a 'double layer' packing mode of the phytanyl surfactants in pairs. $PhyN^+Me_3$ ions, however, may be located preferentially on the outer halflet of the membranes to give the curvature and the large ζ-potentials to the membranes (Table 2) [1].

Many single chain-surfactants in water have been reported to form the micelles, which are not static species but rather exist in a dynamic equilibrium with the surfactant molecules in the bulk aqueous phase [21]. As we could observe by means of TEM, the present assemblies made of equimolecular mixtures of the phytanyl surfactants are static species.

Although the cause of the static nature has not been explained well, it may have arisen from (i) a steric hindrance against desorption of the jagged isoprenoid surfactants from the assemblies and (ii) an ionic attraction between two oppositely charged polar groups. These two terms would cooporate to retard the equilibrium. It may be considered that phytanyl surfactants have inherited the property of forming stable assemblies from the archaebacterial lipids; viz., the isoprenoid lipids furnished the liposomes which were tolerant of such high temperature as 70°C and in such dense NaCl solution as 5M [5–7]. It would be worth noting also that NaCl, protons and proteins were much less mobile in archaebacterial lipid membranes than in the membranes of ordinary glycerophospholipids bearing straight long-chain fatty acids. The low permeability has also been as cried to the steric hindrance between the permeants and the isoprenoid chains. The weak but semistatic assemblies from $C_{16}H_{33}N^+Me_3$ and $C_{15}H_{31}CO_2^-$ (Fig. 2a) would be explained primarily by the term (ii). The destablizing effect of NaCl may be due to dispersion of the ionic interaction by increased ionic strength of the media [22]. Industrial application of the isoprenoid surfactants is under consideration.

[1] The charge repulsion between the same cationic polar heads should be relieved by adsorption of counter anions close to the heads in the Stern layer of the assemblies [20].

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