Biochimica et Biophysica Acta, 1110 (1992) 171–177

BBAMEM 75753

Archaebacterial lipid models: highly salt-tolerant membranes from 1,2-diphytanylglycero-3-phosphocholine

Kiyoshi Yamauchi [a], Kuniyuki Doi [a], Masayoshi Kinoshita [a], Fumiko Kii [b] and Hideki Fukuda [b]

[a] *Department of Bioapplied Chemistry, Osaka City University, Sumiyoshi-ku, Osaka (Japan) and* [b] *Engineering Research Laboratories, Kaneka Co., Miyamae-machi, Takasago-shi, Hyogo (Japan)*

(Received 13 April 1992)

Key words: Liposome; Archaebacterium; Halophile; Permeability; NMR, ^{1}H-; FABMS

1,2-Di(3*RS*,7*R*,11*R*-phytanyl)-*sn*-glycero-3-phosphocholine and its glycerol epimers were synthesized as model lipids of archaebacterial halophiles. These amphiphiles, upon sonication of aqueous suspensions, gave rise to small unilamellar vesicles (SUV) of 300–800 Å in diameter and about 80 Å in the membrane thickness. The liposomes were very stable for at least a month even in a highly concentrated suspension or 5 M aqueous NaCl. The vesicles could retain Na^+ and Cl^- ions as well as 5(6)-carboxyfluorescein in the aqueous interior at temperature as high as 70°C. The liposomes of ordinary diester lipids such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and egg-yolk lecithin were less stable and more permeable than those of the diphytanyl lipids.

Introduction

Archaebacterial halophiles thrive in extreme habitats of such high NaCl concentration (20–30%) as salt lakes and salted foods [1,2]. The cells accumulate salts, mainly in the form of KCl, with a total concentration which is somewhat larger than the level of an external medium. For instance, the internal concentrations of Na^+ and K^+ in *Halobacterium salinarium* were 1.37 and 4.57 M, respectively, when the external concentrations were 4.0 and 0.03 M, respectively [3]. The plasma membranes of the halophiles therefore would be considered to resist free permeation of NaCl and other salts across the membranes. Many of the bacterial lipids so far identified feature the C_{20}- or C_{25}-isoprenoid chains bonded through ether linkages to glycerol (Fig. 1) [4]. By contrast, the membrane lipids of eubacteria and eukaryotes have the fatty acids bonded to glycerol primarily through ester linkages. Assuming that the halophiles possess the unusual lipids in order to cope with the extreme habitat of high salt concentrations, we synthesized 1,2-diphytanyl-*sn*-glycero-3-phosphocholine ((*R*)-DPhyPC) and an epimeric mixture ((*RS*)-DPhyPC) as model lipids (Fig. 2) and examined their liposomal membranes with regard to a barrier property against ions including Na^+ and Cl^- ions as well as stability in high NaCl concentration.

Materials and Methods

DPPC (purity at least 99%) was kindly donated by Nippon Fine Chemical Co. Egg-yolk lecithin was purchased from Sigma (approx. 99%; product No. P 5388). 3*RS*,7*R*,11*R*-Phytanyl bromide was prepared by Raney nickel-catalyzed hydrogenolysis of phytol and the sub-

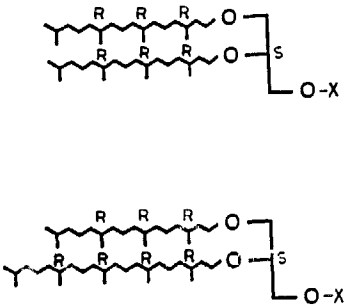

Fig. 1. Halophile lipids. The X residues include phosphoglycerol, phosphosugars, phospho(1-sulfo)glycerol, etc.

Correspondence to: K. Yamauchi, Department of Bioapplied Chemistry, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan.

Abbreviations: CF, 5(6)-carboxyfluorescein; DLS, dynamic light scattering; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; (*RS*)-DPhyPC and (*R*)-DPhyPC, the phospholipids of Fig. 2; FABMS, fast atom bombardment mass spectrum; IR, infrared spectrum; R_f, mobility in TLC; SUV, small unilamellar vesicle; TEM, transmission electron microscopy or micrograph; TLC, thin-layer chromatography; T_m, the temperature at midpoint in gel-to-liquid crystalline phase transition.

sequent bromination of the resulting phytanyl alcohol with HBr-H_2SO_4 (an overall yield, 90%). Thin-layer, column, and gel permeation chromatographies were performed with the supports described previously [5]. Spots in TLC were visualized under ultraviolet light after spraying with 0.25 mM aqueous Rhodamine 6G (for the long chain aliphatic group) and the Dittmer-Lester reagent (for phosphoric acid ester) [6]. The solvent systems in the chromatographies were as follows (system code (volume ratio)): for hexane/ethyl acetate, A (20:1, v/v), B (10:1, v/v), C (5:1, v/v); for chloroform/methanol, D (2:1, v/v); for chloroform/methanol/water, E (65:25:4, v/v); for chloroform/methanol/concentrated ammonia, F (65:35:1, v/v), G (17:35:10, v/v). IR spectra were obtained by means of a Perkin-Elmer FT-IR 1725X spectrometer. ^{1}H-NMR spectra were recorded on Jeol EX-400 spectrometer from a dilute solution in either $CDCl_3$ or a mixture of $CDCl_3$ and CD_3OD (2:1, v/v) with tetramethylsilane as an internal standard. Fast atom bombardment mass spectra (FABMS) were measured using a Jeol HX-100, whereby a sample in a mixture of glycerol on a standard FAB target was subjected to a beam of xenon atoms produced at 8 kV and 2 mA. Ultrasonication was performed with a probe-type ultrasonic disintegrator, Ohtake Works Model 5201. $[Na^+]$ and $[Cl^-]$ ions were determined by the use of a Horiba model H-7LD pH/mV meter, ion-specific electrodes, model 1512A (for Na^+) and model 5002-05T (for Cl^-) in a combination with a model 2535A-06T double junction reference electrode. Other instruments used were a Hitachi H-7000 transmission electron microscope, a Shimadzu RF-502A spectrofluorometer, a Ohtsuka DLS-700 light scattering spectrometer, and a Rigaku DSC-8240 differential scanning calorimeter.

Preparation of DPhyPC(s)

(*R*)- and (*RS*)-DPhyPC(s) were derived from 3-benzyl-*sn*-glycerol and 1-benzylglycerol, respectively. The preparative reactions for the former lipids were the same as those of the latter.

1,2-Di(3RS,7R,11R-phytanyl)glycerol [(RS)-3]. A mixture of sodium hydride (60% in oil, 4.4 g, 0.11 mol) and 1-benzylglycerol ((*RS*)-**1**; 3.63 g, 20.1 mmol) in dry tetrahydrofuran (THF; 85 ml) was stirred at room temperature for 1 h. 3*RS*,7*R*,11*R*-Phytanyl bromide (17.7 g, 49.0 mmol) was added to the resulting alkoxide solution, and the mixture was heated in an oil bath of 145°C for 4 h; the solvent was distilled out during an initial stage of the heating. To the residue was added the phytanyl bromide (3.2 g, 8.8 mmol), and heating at the temperature was continued for another 2.5 h. The reaction mixture was fractionated between chloroform and water. The organic layer was dried with anhydrous sodium sulfate, concentrated and applied to a silica gel column. The compound [(*RS*)-**2**] was eluted by solvent A; a colorless oil, 8.36 g (57%); IR (neat): 2952, 2924, 1495, 1462, 1377, 1366, 1102 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.836–0.874 (m, 30 H, 10 CH_3), 0.969–1.364 (m, 44 H), 1.441–1.690 (m, 4 H), 3.442–3.634 (m, 9 H, $-CH_2OCH_2CH(OCH_2-)CH_2O-$), 4.498 and 4.551 (s, 2 H, $CH_2C_6H_5$), 7.168–7.365 (m, 5 H, C_6H_5); FABMS (Xe; matrix, glycerol): *m/z* (relative intensity) 743 ($(M+1)^+$, 45).

Next, to a suspension of 5% palladium on carbon (Aldrich, 1.49 g) in ethanol (60 ml) containing hydrochloric acid (0.3 ml) was added (*RS*)-**2** (7.32 g, 10.0 mmol), and the mixture was stirred under atmospheric pressure of hydrogen at room temperature for 4 h. After removal of the catalyst by filtration with Celite, the filtrate was concentrated and applied to a silica gel column. Elution with hexane/ethyl acetate (from 5:1 to 2:1 v/v) gave (*RS*)-**3** as oil, 4.91 g (77%); R_f (solvent C) 0.56 (*S*-**3**), 0.40 (*R*-**3**); IR (neat): 3418, 2952, 2924, 2866, 1462, 1377, 1366, 1116, 1057cm^{-1}; ^{1}H-NMR: δ 0.837–0.903 (m, 30 H, 10 CH_3), 1.048–1.383 (m, 44 H), 1.475–1.674 (m, 4 H), 3.425–3.741 (m, 9 H, $-CH_2OCH_2CH(OCH_2-)CH_2-$); FABMS (Xe; matrix: glycerol): *m/z* (relative intensity) 652 ($(M+1)^+$, 35).

Debenzylation of **2** could be performed also by a reaction with ethyl mercaptan-BF3 reagent. Namely, (*R*)-**2** (0.76 g, 1.02 mmol), which was dried upon azeotropic distillation with benzene, in tetrachloromethane (20 ml) was mixed with boron trifluoride etherate (3.0 ml) and ethyl mercaptan (3.0 ml). After standing at room temperature overnight, the solution was neutralized by 10% aqueous sodium hydroxide, and concentrated by passing nitrogen gas through the solution which was warmed by means of a water bath (the procedure was conducted in a draft chamber). A chloroform solution of the residue was washed with a saturated aqueous solution of sodium chloride, dried on anhydrous sodium sulfate and concentrated. Column chromatography of the residue using the solvent C afforded (*S*)-**3** as colorless oil, 0.44 g (66%); R_f 0.56 (solvent C); $[\alpha]_D^{20}$ −7.0° (*c* 21, chloroform). The product showed IR and FABMS spectra which were essentially similar to those of the *RS*-isomer.

1,2-Di(3RS,7R,11R-phytanyl)-sn-glycero-3-phosphocholine[(R)-DPhyPC)]. 1,2-Diphytanyl-*sn*-glycerol ((*S*)-**3**, 0.33 g, 0.51 mmol), which was dried by azeotropic distillation with benzene, was stirred in the presence of (2-bromoethyl)phosphorodichloridate (1 ml) at about 110°C for 40 min. The cooled reaction mixture was agitated with water (5 ml) for a while and allowed to stand in a refrigerator overnight. The resulting solid was washed with cold water, dried, and applied to a silica gel column. Elution with the solvent F gave the lipid precursor [(*R*)-**4**] in the fraction which was positive to both Dittmer-Lester reagent and Rhodamine 6G sprays, 0.32 g (75%); R_f 0.66 (solvent F), m.p.

164–167°C. Next, a mixture of (*R*)-**4** (0.18 g, 0.22 mmol) and 2.4 M aqueous trimethylamine (20 ml) was warmed in a sealed container at 50°C for 50 h, then concentrated and applied to a silica gel column using solvent G. The fraction, which gave a homogeneous spot upon thin-layer chromatography, was further purified by means of a gel column (Sephadex LH-20; 2 cm × 60 cm). Elution with the solvent D gave (*R*)-DPhyPC as colorless solid, which was freeze-dried from its benzene/methanol(trace) solution; 0.16 g (88%); m.p. 176–180°C; R_f 0.58 (solvent E); $[\alpha]_D^{20}$ 12.2° (*c* 21, chloroform/methanol, 2:1, v/v); ^{1}H-NMR ($CDCl_3$/CD_3OD, 2:1, v/v): δ 0.846–0.890 (m, 30 H, 10 CH_3), 1.087–1.377 (m, 44 H), 1.516–1.549 (m, 4 H), 3.215 (s, 9 H, $N(CH_3)3$), 3.36–3.39 (m, 2 H), 3.43–3.50 (m, 2 H), 3.60 (br, 2 Hh), 3.64 (br, 2 H), 3.91 (t, 2 H), 4.3 (br, 2 H, CH_2N); FABMS (Xe; matrix; glycerol): *m/z* (relative intensity) 818 ($(M + 1)^+$, 28).

Found: C, 66.30; H, 13.09; N, 1.98%. Calcd. for $C_{48}H_{100}O_6NP \cdot 3\ H_2O$: C, 66.09; H, 12.25; N, 1.61%.

(*RS*)-DPhyPC was prepared from (*RS*)-**3** by performing reactions similar to those above; m.p. 184–207°C (measured by DSC); the product exhibited two spots (R_f 0.58 and 0.24) in TLC (solvent E) and two singlet peaks at δ 3.215 and 3.223 [for $(CH_3)_3N$] in the ^{1}H-NMR ($CDCl_3$/CD_3OD, 2:1, v/v). The underlined values belong to the (*R*)-DPhyPC and the values without an underline to the (*S*)-isomer.

Liposomes

The lipid in distilled water or 0.05 M Tris-HCl (pH 7.8) was sonicated at 10°C (for DPhyPC(s)) or 50–55°C (for DPPC and egg-yolk lecithin) at 30–40 W for 25–30 min. The ratio of the amphiphile to the solvent was 1–3 mg/ml. The resulting suspension was centrifuged at 2000 × *g* for 15 min to give the supernatant, which was then subjected into transmission electron microscopic observation using 1 wt% aqueous phosphotungstic acid/sodium hydroxide (pH 7) as a staining agent. Specimens were prepared according to a previously described procedure [7]. A typical micrograph of the liposomes of (*R*)-DPhyPC is displayed in Fig. 3.

Differential scanning calorimetry

(*R*)- and (*RS*)-DPhyPC (10.5 mg) in water/glycerol (80:20 w/w; 1.0 g) were vortexed at room temperature. The resulting dispersions (about 20 mg) in an aluminum pan were weighed accurately, then examined using a highly sensitive calorimeter. Temperature was scanned from −40 to 80°C. Approximately equal weight of the antifreeze solvent was placed in a reference pan.

Leakage of CF from liposomes

(A) Temperature effects. A mixture of lipid ((*R*)-DPhyPC and other lipids such as (*RS*)-DPhyPC, 5 mg) and 0.2 M CF in 0.05 M Tris-HCl buffer (pH 7.8) (1 ml) was sonicated at 50°C for 25 min. The resulting dispersion was centrifuged at 2000 × *g* for 15 min to give the supernatant, which was applied to a gel column (2 cm × 25 cm; Sephadex G-50 (fine)) equilibrated with the same buffer. The SUVs bearing the fluorescent probes were eluted first (1.5–2 ml) and separated from free probe. The apparent concentration of CF in an aqueous interior of the liposomes was estimated to be 0.17–0.2 M on the basis of a quantitative phosphorus assay (for the lipid) and UV absorption at 495 nm (for the probe) of the SUV suspension, assuming that the diameter of the liposomes was 600 Å in average (see TEM and DLS in Fig. 3) and a limiting area of the DPhyPC in the membrane was 90 Å^2 by the Langmuir surface area vs. pressure isotherm (the data are not shown). A separate experiment showed that fluorescent emission of CF was quenched completely above 10 mM.

The SUV solution (30 μl) was diluted with the same buffer to 3 ml in a quartz cell, and the fluorescence intensity (I_t) at 520 nm with excitation at 495 nm (band width: excitation, 10 nm; emission, 5 nm) was measured at a temperature range of 5–70°C. The temperature of the cell was controlled within ±0.5°C by circulating warm water in the cell holder. After the measurement, the sample solution was mixed with 10 vol% aqueous Triton X-100 solution (10 μl), and the maximum intensity (I^*) resulting from instantaneous rupture of the liposomes was measured. The extent of leakage (I_t/I^*) observed after equilibration of temperature was plotted against temperature as seen in Fig. 4. All of the fluorescent intensity data were calibrated to the value of 25°C by use of a calibration curve for temperature effects on intensity.

(B) Salt effects. Liposome (SUV) suspensions containing 0.2 M CF or a mixture of 0.2 M CF and 3 M NaCl in the interior were prepared in a procedure similar to that mentioned above. The suspensions were diluted by 100 volumes with aqueous NaCl of various concentrations, allowed to stand for 10 min, then the fluorescence intensity was recorded. Using calibration curves of the fluorescence intensity vs. [NaCl], the amounts of CF leaked from the damaged vesicles were estimated. All of the operations were carried out at 20°C.

Leakage of Cl^- and Na^+ ions from liposomes

Lipid ((*R*)-DPhyPC and other lipids such as (*RS*)-DPhyPC, 15 mg) was sonicated in 1.0 M sodium chloride (3 ml) at 50°C and at 30 W for 25 min. The suspension was centrifuged to give the supernanant, which was then applied to a gel column (Sepadex G-50 (fine), 2 cm × 25 cm)) equilibrated with 1.0 M sodium nitrate. Elution with 1.0 M sodium nitrate gave the liposomes containing sodium chloride in the liposome interior with an isoosmotic external medium (= 1.8

osmol/kg) [8]. 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.

Measurement of the leakage of Na^+ ions was attempted in a similar manner using liposome-suspensions having 1.0 M NaCl in the interior and isoosmotic 1.6 M aqueous ethylene glycol in the external medium.

Results

Synthesis of (R)- and (RS)-DPhyPC

The model lipids were synthesized by a series of the reactions displayed in Fig. 2. In the rather classical strategy, we attempted to promote efficiency in the etherification and phosphorylation steps of the glycerol hydroxyl groups of **1** and **3**, respectively; and in consequence, the conversion of **1** into the diphytanyl ether (**2**) and (2-bromoethyl)phosphorylation of **3** into the lipid precursor (**4**) could be carried out in good yields (about 57 and 76%, respectively) upon heating the reaction mixtures at 100–140°C in the absence of any solvents. The overall yield of DPhyPC(s) from **1** was 15–29%.

Liposomes

Sonication of (*R*)- and (*RS*)-DPhyPC in aqueous media such as pure water and 0.05 M pH 7.8 Tris-HCl buffer gave quantitatively clear suspensions of SUVs of 300–900 Å in diameter and about 70 Å in membrane thickness (Fig. 3). The liposome suspensions were very stable at ambient temperature for at least one month as judged by similar properties in TEM and DLS. Even a dense suspension containing > 7 mg of lipid/ml did not show any morphological change over that period.

The liposomes of DPPC and egg-yolk lecithin were approximately similar in diameter and membrane thickness to the vesicles of DPhyPC(s). However, unlike the liposomes of DPhyPC(s), those liposomes of the ordinary lipids aggregated readily (precipitated in the case of DPPC liposomes) when the suspensions were highly populated with vesicles (> 5 mg of lipids per 1 ml of an aqueous medium).

DSC study

It was found that the membranes of the DPhyPC(s)-liposomes did not undergo a gel-to-liquid crystalline phase transition over a temperature range from −40 to 80°C. An absence of the phase transition has also been reported in DSC studies dealing with archaebacterial lipids and model compounds [9–11].

Barrier properties

Both (*R*)- and (*RS*)-DPhyPC lipids furnished liposome membranes which functioned as good barriers against leakage of CF as well as Na^+ and Cl^- from low to high temperature (0–70°C) (Figs. 4–6). By contrast, the liposome membrane of DPPC (T_m = 42°C) became leaky at temperature above about 30°C and especially at the T_m. The liposomes made of egg-yolk lecithin (T_m = -15°C) were only good as a container at temperature up to about 40°C.

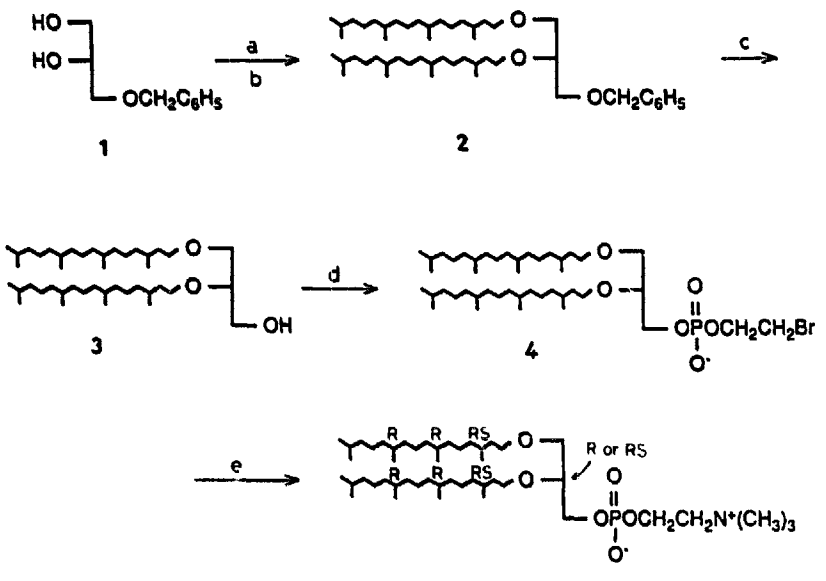

Fig. 2. Synthesis of DPhyPC(s). (a) NaH in tetrahydrofuran, (b) (3*RS*,7*R*,11*R*)-phytanyl bromide, (c) Pd/C in 0.5% HCl/EtOH or EtSH-BF_3·OEt_2, (d) (2-bromoethyl)phosphorodichloridate, (e) aqueous trimethylamine. The *sn*-2 carbon of **1**–**4** and DPhyPC(s) are either *R*- or *RS*-conformation; the compounds are hence denoted by the superscripts; for instance, (*R*)-**1** or (*RS*)-**1**, etc.

Membrane stability in aqueous NaCl

It seemed from Fig. 7 that the liposomes of egg-yolk lecithin burst when the vesicles were exposed to above about 1.5 M NaCl, permitting all CF molecules to escape from the interior within 10 min (■ line). The liposomes made of DPPC were also considerably fragile in the salt solution (● line), and the damage to the trapping ability was increasingly significant with increasing NaCl concentration. By contrast, the liposomes of (*RS*)-PhyPC were much more salt-tolerant and retained most CF molecules even in 5 M NaCl (○ line); the (*R*)-PhyPC also gave vesicles of a similar

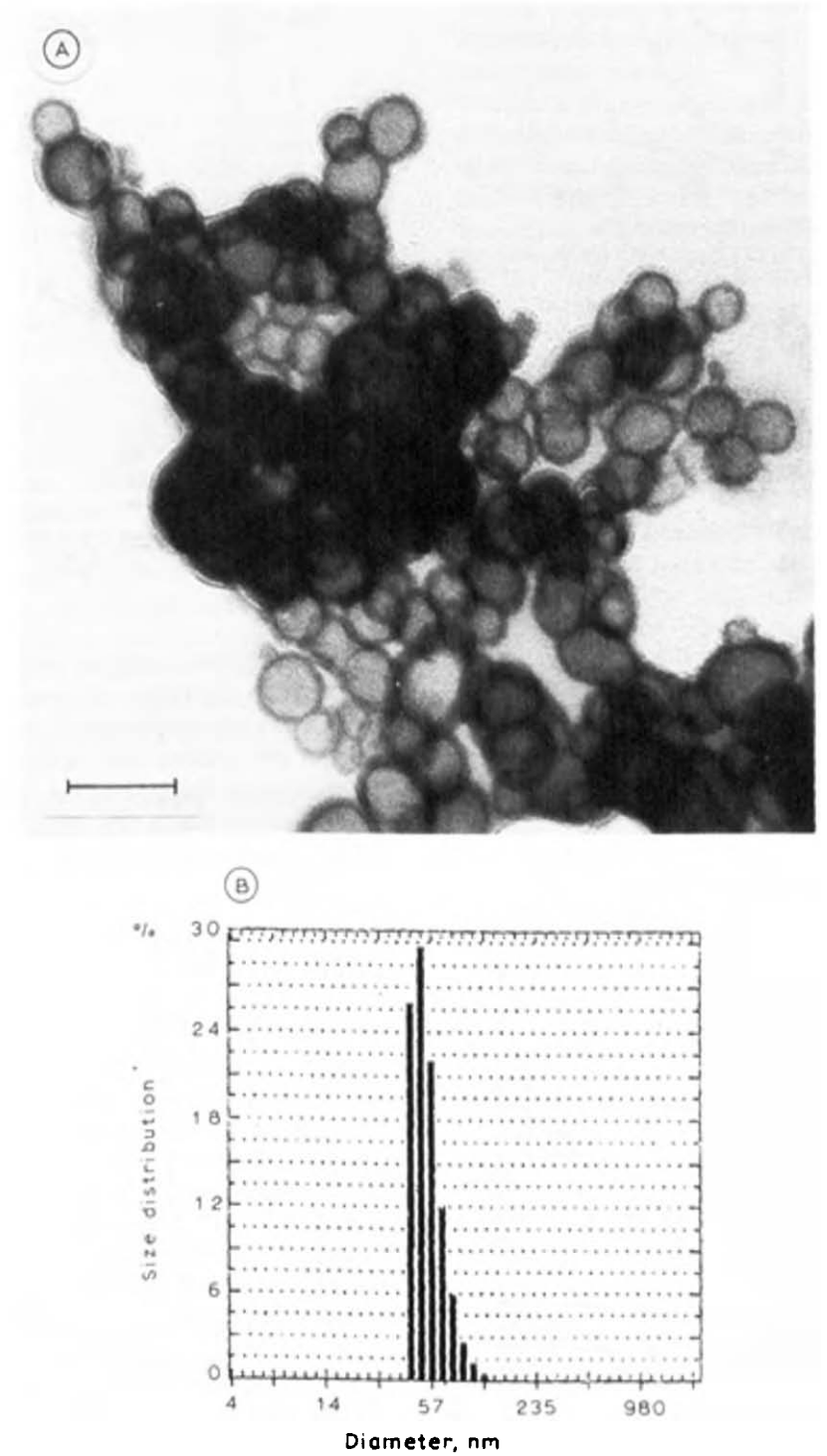

Fig. 3. Transmission electron micrograph of the liposomes of (*R*)-DPhyPC; negative staining with phosphotungstic acid/sodium hydroxide. The bar is 1000 Å. The histogram of the diameter distribution (number average) was obtained by DLS on an aqueous suspension of 5 days after sonication. The liposome suspensions used for the TEM and DLS studies were not processed by gel permeation chromatography.

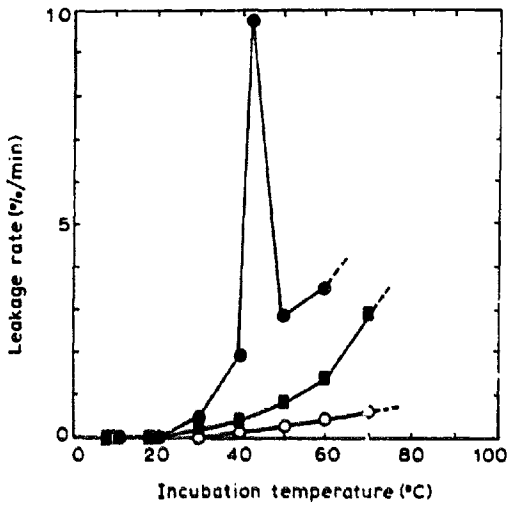

Fig. 4. Initial leakage extent (%) of CF per min from the liposomes of DPPC (●), egg-yolk lecithin (■) and (*R*)-DPhyPC (○) as a function of temperature; solvent, 0.05 M Tris-HCl (pH 7.8).

resistance to leakage in the salt solutions (the data are not shown).

On the other hand, it appeared that all of the liposomes, including those prepared from DPPC and egg-yolk lecithin, were insensitive to osmotic pressure; viz., they retained CF molecules inside the vesicles even when the vesicles having 2 M NaCl in the interior were exposed to plain water as shown in Fig. 8. The results agree with the previous observation that liposomes were generally stable to osmotic strength caused by small molecules such as NaCl [12, 13].

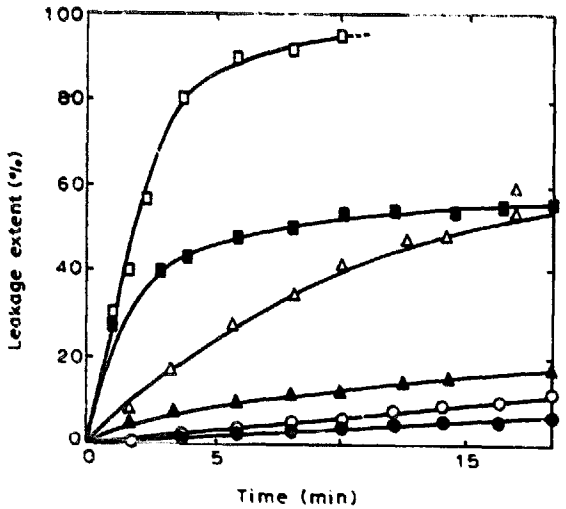

Fig. 5. Leakage extent of Cl^- ions from the liposomes of DPPC (■, 20°C; □, 42°C); egg-yolk lecithin (▲, 20°C; △, 42°C); (*R*)-DPhyPC (●, 20°C; ○, 42°C) as a function of incubation time. Solvents: 1 M NaCl (inside), 1 M $NaNO_3$ (outside).

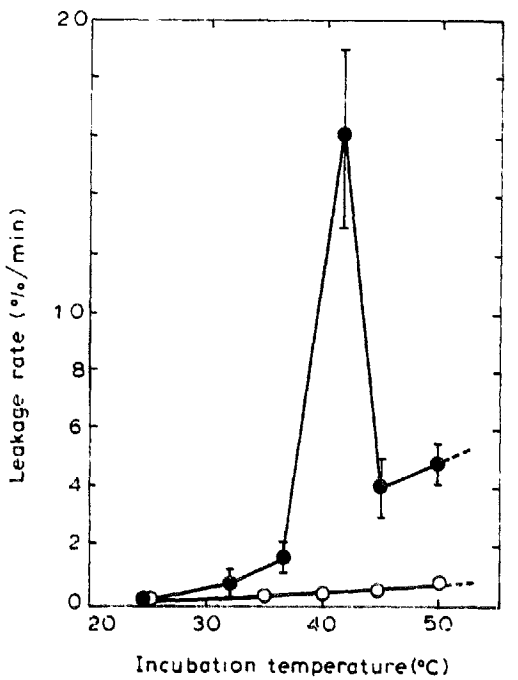

Fig. 6. Initial leakage extent (%) of Na^+ ions per min from the liposomes of DPPC (●) and (*R*)-DPhyPC (○) as a function of temperature. Solvents: 1 M NaCl (inside), 1.63 M ethylene glycol (outside).

Discussion

The liposomes made of ordinary ester-type lipids are prone to aggregate and precipitate, especially when the vesicles are concentrated in suspension or they are brought into contact with metal ions, or both. Most of the substances trapped in the interior are released to the exterior within a few days. For instance, SUV of DPPC, which appeared stable in the dilute suspension,

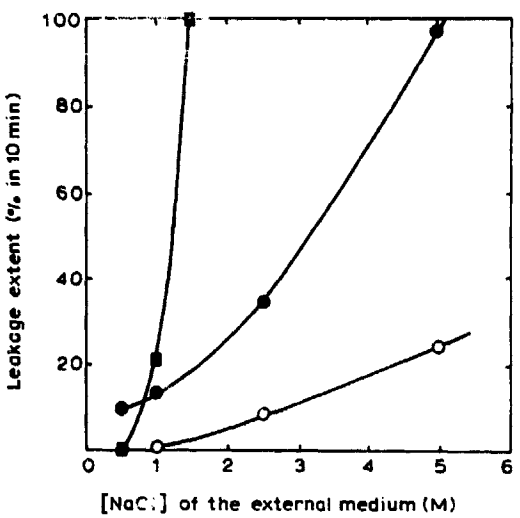

Fig. 7. Leakage of CF molecules through a breakdown of liposomes of DPPC (●), egg-yolk lecithin (■) and (*RS*)-DPhyPC (○) in aqueous NaCl at ambient temperature (20–25°C); solvents: 0.2 M CF (inside), from 0.5 to 5 M NaCl (outside).

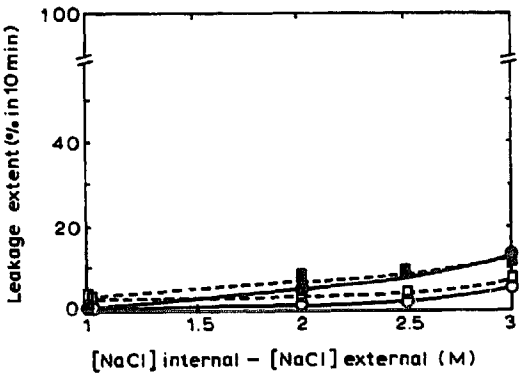

Fig. 8. Leakage of CF molecules through the liposomes of DPPC (15°C, ■; 30°C, □), and (*RS*)-DPhyPC (15°C, ●; 30°C, ○) in aqueous NaCl; solvents: the aqueous interior nad 0.2 M CF and 3 M NaCl, and [NaCl] of the bulk exterior was varied from 0 to 2 M.

permitted 10–20% of total CF moleclues to escape during the first 10 min of incubation at 40°C (Fig. 4); and a thick vesicle suspension (> 5 mg/ml of water) was unstable and gave precipitates upon standing at ambient temperature within a day.

On the contrary, aqueous suspensions of (*R*)- and (*RS*)-DPhyPC liposomes were stable even in a concentrated state as judged from (i) the absence of any precipitates and (ii) the DLS measurements, which provided the the diameter distribution similar to Fig. 3 over a month or more at ambient temperature. The membranes were much more resistant to permeation of CF as well as Na^+ and Cl^- ions over a wide temperature range (0–70°C) (Figs. 4–6) and resisted effects of high external and internal concentrations of NaCl (Figs. 7 and 8).

The results presented here would substantiate not only a highly salt-tolerant property but also thermostability of halophile membranes. Although these properties have not been explained well, the stability would originate likely from the presence of the isoprenoid chains since the liposomes of DPhyPCs possess the same phosphocholine interfacial group as those of DPPC and egg-yolk lecithin. NMR study of natural halophile lipids suggested that despite their being in a highly fluid state the segmentary motion at the tertiary carbons was hindered due to the presence of the methyl side groups [11,14]. Thus, the isoprenoid chain arrays may interact strongly in the membranes causing greater resistance to the diffusing CF and the inorganic ions such as Na^+ and Cl than the n-alkyl and alkenyl fatty acid-chains of phosphatidylcholines. It has been shown that archaebacterial tetraetherlipids having C_{20}- and C_{40}-isoprenoid chains gave also the liposomal membranes which were much less permeable for CF and polyols such as glycerol than the membranes prepared from ordinary lipids [15,16].

In the above (*R*)-DPhyPC, the phytanyl groups are a mixture of four diastereomers, and the natural lipid-chains are composed of only one diastereomer: 3*R*,7*R*,11*R*-phytanyl group. We have had some difficulty with chemical synthesis of the optically pure phytanyl segment [17,18]. Hence, the problem whether the effects of the mixture of the stereoisomers are the same or not with one diastereomer has been unsettled. Finally, it should be noted that the DPhyPCs furnished always liposomes in a quantitative yield over a wide temperature range (5–70°C). We did not observe a hexagonal H_{II} phase despite the relatively large ratio of hydrophobic part/hydrophilic part of the DPhyPCs, which was about twice that of ordinary diester lipids such as DPPC [19,20]. The morphology of molecular assemblies of various halophile lipids and the biocompatible use of the membranes are still under study.

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