Archaebacterial Lipids: Surface Pressure–Surface Area Isotherms of 1,1′-(1,32-Dotriacontanediyl)bis[2-[(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl]-*sn*-glycero-3-phosphocholine]

Kiyoshi YAMAUCHI,* Katsu YAMADA, Masayoshi KINOSHITA, and Tadao KAMIKAWA†
Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, Sumiyoshi-ku, Osaka 558
† Department of Chemistry, Faculty of Science and Technology, Kinki University, Kowakae, Higashiosaka 577
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1,1′-(1,32-Dotriacontanediyl)bis[2-[(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl]-*sn*-glycero-3-phosphocholine] (the tetramethylhexadecyl group=phytanyl) was synthesized conveniently by reactions involving etherification of 1,1′-(1,32-dotriacontanediyl)bis(3-benzyl-*sn*-glycerol) (**1**) with (7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl bromide and hydrogenation of the resulting unsaturated ether with *p*-tolylsulfonylhydrazine; a total yield from **1**, 7—10%. Unlike usual monopolar double-chain-amphiphiles, the bipolar lipid bent into a U-form in a water–air interface to produce the Langmuir membrane, exhibiting peculiar surface pressure–surface area isotherms.

Previously we mentioned the highly thermostable liposomes made of 1,1′-(1,32-dotriacontanediyl)bis[2-[(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl]-*sn*-glycero-3-phosphocholine] (the tetramethylhexadecyl group=phytanyl) (**L-32-Phy**).[1] The membrane, despite its low gel-to-liquid crystalline phase temperature (T_m; 8 °C), could retain well not only small molecules such as 5-(and 6-)-carboxyfluorescein (MW 376) but also large phospholipase A_2 (MW 14000) at high temperature as 80 °C. The bipolar lipid, however, was obtained via a multistage synthetic pathway in a total yield of 3—8%. In this paper we wish to describe an improved preparation of **L-32-Phy** and surface pressure–surface area isotherms of the amphiphile in an interface between water and air.

Experimental

1,2-Distearyl-*sn*-glycero-3-phosphocholine (DSPC) was prepared previously[2]; cf., the "stearyl" means "octadecyl". Thin-layer, column and gel permeation chromatographies were performed using the supports mentioned previously.[1] Compound spots in TLC were visualized by spraying with 0.0012% Rhodamine 6G and the Dittmer–Lester reagent.[3] IR spectra were measured by the use of a JASCO A-100 spectrometer. ^{1}H NMR spectra were recorded on JEOL PS-100 and GX-400 spectrometers using a dilute solution in $CDCl_3$ and tetramethylsilane as an internal standard. Fast atom bombardment mass spectra (FABMS) were obtained using a JEOL HX-100, whereby a sample in a matrix was subjected to a beam of xenon atoms produced at 8 kV and 2 mA. Optical rotation was determined by means of a JASCO DIP-360 digital polarimeter. A microprocessor-controlled film balance, Sun-etsu FDS-20, was utilized for the monolayer study.

(7*R*,11*R*)-3,7,11,15-Tetramethyl-2-hexadecenyl bromide (2). Phosphorus tribromide (1.35 g) in anhydrous hexane (2 ml) was added dropwise to the solution of isophytol (2.97 g, 0.01 mol), pyridine (0.4 ml) and hexane (6 ml), which was stirred and cooled to −7—−10 °C. After addition, the mixture was stirred at the temperature for 3 h, then poured into ice-water and extracted with diethyl ether. The organic extract was washed with 1 M hydrochloric acid (1 M=1 mol dm^{-3}), then with aqueous sodium hydrogencarbonate and dried by anhydrous magnesium sulfate. Upon removal of the solvent, the bromide (**2**) was obtained as oil, 3.56 g (99%); mobility (R_f) in TLC (hexane) 0.6; IR (neat) 2960 (s), 1655 (w), 1465 (s), 1380 (m), and 1200 (m) cm^{-1}; ^{1}H NMR (100 MHz) δ=0.87 (d, 12H, *J*= 7 Hz, 4CH_3), 1.25 (m, 19H, CH and CH_2), 1.70 (d, 3H, *J*= 1 Hz, *trans*-CH_3), 2.00 [t, 2H, *J*= 7 Hz, $C\underline{H}_2(CH_3)CH{=}CH$], 4.05 (d, 2H, *J*= 8 Hz, CH_2Br), and 5.56 (t, 1H, *J*= 8 Hz, $C{=}C\underline{H}CH_2Br$). Although the ^{1}H NMR spectrum showed also the minor signals attributable to impurities, the bromide was used for the next reaction without further purification since it changed to unknown(s) when distillation was attempted under reduced pressure (bp about 145—155 °C/3 mmHg, 1 mmHg= 133.322 Pa).

1,1′-(1,32-Dotriacontanediyl)bis[2-[(7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl]-3-benzyl-*sn*-glycerol] (3). 1,1′-(1,32-Dotriacontanediyl)bis(3-benzyl-*sn*-glycerol)[4] (**1**; 1.78 g, 2.07 mmol) and sodium hydride (60% in oil, 0.34 g, 8.1 mmol) in anhydrous tetrahydrofuran (25 ml) were stirred at room temperature for 1 h. Compound (**2**, 3.0 g, 8.2 mmol) was added to the resulting alkoxide solution. After heating to reflux for 20 h, the reaction mixture was concentrated, fractionated between water and chloroform. The organic extract was subjected to a silica-gel column chromatography using hexane–ethyl acetate (25/1 v/v). The fraction, which showed a homogeneous spot in TLC under the UV-light and with the Rhodamine 6G spraying, gave **3** as colorless viscous liquid, 2.0 g (71%); R_f (hexane–ethyl acetate, 10/1 v/v) 0.40; 100 MHz ^{1}H NMR δ=0.83—0.87 (a mixture of s and d, 30H, 10CH_3), 4.15 (d with a complex line pattern, 4H, $=CHC\underline{H}_2$), 4.5 (s, 4H, $C\underline{H}_2C_6H_5$), 5.35 (s with a complex line pattern, 2$\underline{H}$, $C\underline{H}{=}CH_2O$) and 7.32 (s, 10H, 2C_6H_5); positive FABMS (matrix: 3-nitrobenzyl alcohol and NaCl) *m*/*z* (rel intensity) 1389 (M+Na, 3%), 1366 (M^+, 1%).

1,1′-(1,32-Dotriacontanediyl)bis[2-[(3*RS*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl]-3-benzyl-*sn*-glycerol] (4). A diglyme solution (15—20 ml) of **3** (1.79 g, 1.3 mmol) and *p*-tolylsulfonylhydrazine (2.5 g, 13 mmol) was heated to reflux under nitrogen for 3 h. The reaction mixture was concentrated to about 10 ml, diluted with hexane and, after removal of the resulting precipitates, was subjected into silica-gel column chromatography. Elution with hexane–ethyl acetate (15/1

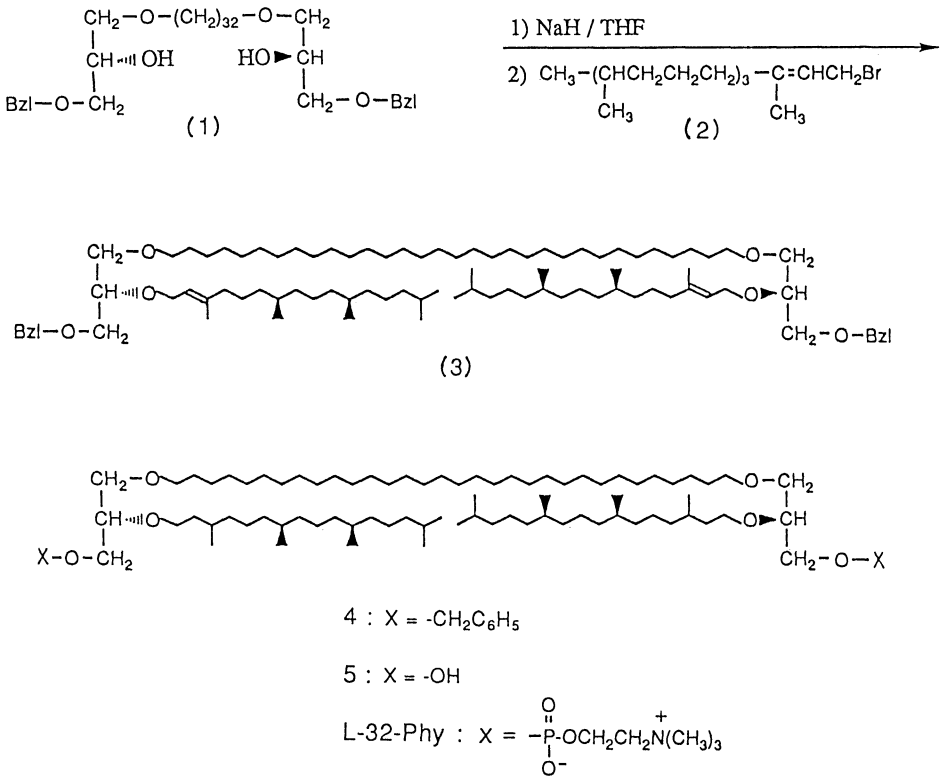

Fig 1.

v/v) gave the diglycerol (**4**) which was further purified by means of a Sephadex LH-20 column using chloroform–methanol (2/1 v/v) as an analytically pure compound, 1.3 g (73%); colorless oil; R_f (hexane–ethyl acetate, 10/1 v/v) 0.56; $[\alpha]_D^{25}$ +1.1° (*c* 1.0, chloroform); 100 MHz ^{1}H NMR and IR spectra were identical with that of the authentic sample;[1] FABMS (matrix: glycerol/3-mercapto-1,2-propanediol, 1/1 v/v) *m/z* (rel intensity) 1371 (M+H, 10%).

L-32-Phy. The dipolar lipid was obtained from **4** via the debenzylation and phosphocholination reactions which were described previously;[1] an overall yield from **4**, 13—18%; mp 152—158 °C (lit,[1] 152—160 °C); a mobility in TLC as well as ^{1}H NMR and IR spectra were identical with those of an authentic sample.

Surface Pressure–Surface Area Isotherms. The film balance system consisted of a Teflon-coated trough of 506 mm (length)×150 mm (width) and a microprocessor (NEC, PC-9801). Temperature of the subphase (water) was thermostated with circulating water within the aluminum trough. A benzene–ethanol solution of **L-32-Phy** (45 µl; 2.66 mg ml^{-1} of the solution) was placed gently on the surface of the subphase, then, a Teflon barrier was swept to compress the film at speed (1 point s^{-1} or 60 mm^2 s^{-1}). The pressure (π) was calibrated by the π–area curve of stearic acid monolayer. The typical results are shown in Fig. 1.

Results and Discussion

In the previous preparation of **L-32-Phy**, phytanyl-etherification of **1** at the *sn*-2- and -3-O atoms was the most inefficient step; viz., **3** was obtained in a yield of only 15—20% after refluxing a THF-suspension of the sodium salt of **1** and phytanyl bromide for 84 h. The present procedure utilized, instead of the saturated bromide, (7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl bromide (**2**). The allylic bromde was reactive to the alkoxide of **1** to produce **3** in a yield of as much as 72%. The reaction time was also shortened considerably. ^{1}H NMR as well as EIMS and FABMS spectra were in accordance with the assigned structure displaying the signals for the C(CH$_3$)=CH–CH$_2$O moiety.

Conversion of **3** into **4** was attempted using various reducing reactions. Palladium-catalyzed hydrogenation of **3** was not fruitful. Heating of **3** with *p*-tolylsulfonylhydrazine[5] in diglyme was efficient to afford **4** in a yield of 73%. Conversion of **4** into **L-32-Phy** was performed according to the previous procedure;[1] namely, (i) debenzylation of **4** by the use of BF$_3$·O(C$_2$H$_5$)$_2$/ethanethiol, (ii) phosphocholination of the resulting diglycerol (**5**) with 2-bromoethyl phosphorodichloridate and trimethylamine. A total yield from **1**: 7—10% in the present method.

Now, the bipolar **L-32-Phy** was different in surface pressure (π)–surface area isotherm from DSPC, one of typical monopolar amphiphiles (Fig. 2). Namely, DSPC gave a stable Langmuir membrane with a limiting area of about 50 Å^2/molecule (curves 1—3). The surface pressure raised sharply upon compressing the spreading area and collapsed at a high lateral pressure as 63 mN m^{-1}. The breakdown occurred instantaneously at an apparent surface area about 36 Å^2/molecule. By

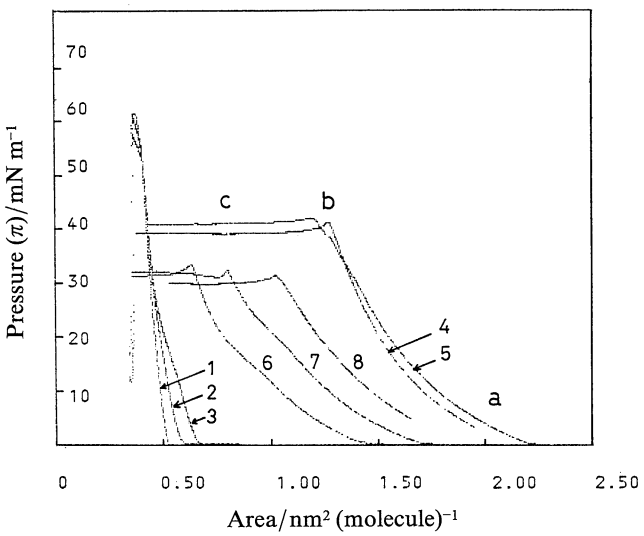

Fig. 2. Surface pressure (π)-surface area isotherms; subphase=water. **DSPC**: curve 1 (5 and 10°C), 2 (15°C), and 3 (20°C); **L-32-Phy**: curve 4 (5°C), and 5 (15°C) (the isotherm curve of 10°C was omitted); **L-32-16**: curve 6 (5°C), 7 (10°C), and 8 (20°C).

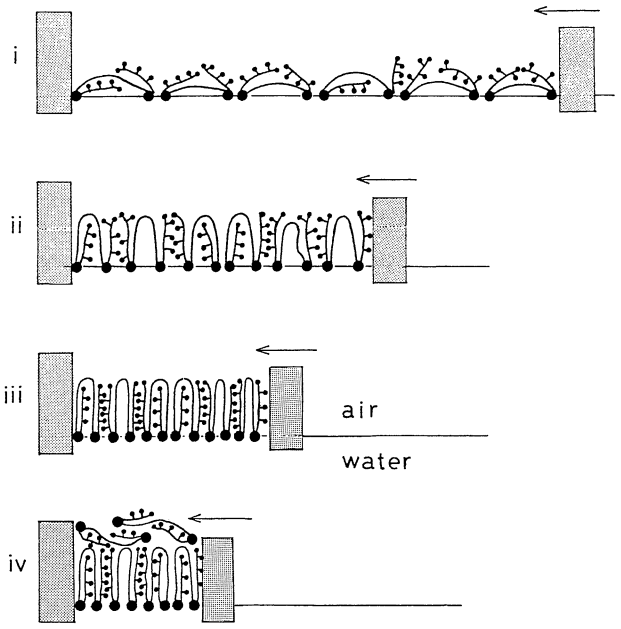

Fig. 3. Schematic representations of formation and collapse of **L-32-Phy** in an interface between water and air.

contrast, **L-32-Phy** did not exhibit a highly cooperative compression; viz., the surface pressure increased gradually upon compressing the spreading area (curves 4 and 5). The limiting area was as large as 160 Å^2/molecule. It appeared also that the monolayer did not collapse, holding constant pressure as inferred by the parallel lines with the abscissa (from point b through c). The peculiar isotherms of **L-32-Phy** may be rationalized as follows. At the low pressure, the bipolar lipids lie sporadically on a water/air interface with the polar heads in water and the hydrophobic residues in air (Fig. 2, point a; Fig. 3-i). With a continuing sweep of the barrier of a film balance, the bipolar lipids are bent gradually to a U-form interacting with neighboring bipolar lipids until the limiting area is closed to the value calculated for two phosphocholine heads (Fig. 2, point b; Fig. 3-ii and iii); cf. DSPC, and DOPC; 50 (this study) and 72 Å^2/molecule,[6] respectively. Upon further compression, the U-shaped **L-32-Phy** may be extruded from the monolayer to pile up on the membrane; the process may permit the constant surface pressure (Fig. 3-iv).

On the other hand, we dealt previously with a Langmuir membrane of 1,1′-(1,32-dotriacontanediyl)-bis(1,2-hexadecyl-*sn*-glycero-3-phosphocholine) (**L-32-16**).[4] The bipolar lipid bearing slim hexadecyl groups gave the π–area isotherms (Fig. 2, curves 6—8) which were characterized by (*a*) significant temperature-dependency as seen in the varying limiting area (90, 120, and 160 Å^2/molecule at 5, 10, and 20 °C, respectively; cf., T_m=61.5 °C; ΔS=0.8 eu/methylene unit) and (*b*) low maximum collapsing pressure (about 30 mN m^{-1}). By contrast, **L-32-Phy**, despite of the low T_m (8 °C),[1] showed that (*a′*) the π–area isotherm or the limiting area was relatively insensitive to temperature (5—15 °C), and (*b′*) the Langmuir membrane was more stable mechanically than the **L-32-16** membrane as judged from the relatively large collapsing pressure (about 40 mN m^{-1} in Fig. 2). Though the above aspects (*a′* and *b′*) have not been fully explained, the temperature-insensitivity might be explained by the ΔS in the phase transition which was as small as 0.4—0.6 eu/methylene unit or high fluidity of the phytanyl groups,[7] and the mechanical stability might be ascribed to the branched structure which would offer high frictional resistance between hydrophobic chains in the membrane. It, however, was apparent from the maximum collapsing pressure that a double-chain-monopolar amphiphile such as DSPC produce a more stable Langmuir membrane than **L-32-Phy**.

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