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Hydroxyarchaetidylserine and hydroxyarchaetidyl-*myo*-inositol in *Methanosarcina barkeri*: polar lipids with a new ether core portion

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Key words: Ether lipid; Hydroxyarchaeol; Archaebacteria; Serine lipid; Inositol lipid; (*M. barkeri*)

Lipids of the methanogenic archaebacterium, *Methanosarcina barkeri* were analyzed. The lipid content was 5.4% of dry cell and polar lipids comprised 87% of the total lipid. Polar lipids were separated into 14 spots by two-dimensional thin-layer chromatography. These were six phospholipids, seven aminophospholipids and one glycolipid, of which two phospholipids and two aminophospholipids were major constituents. After removal of polar head groups from total lipids, two kinds of glycerol diether core lipids were found. One was 2,3-di-*O*-phytanyl-*sn*-glycerol (archaeol) and the other 2-*O*-(3′-hydroxy-3′, 7′, 11′, 15′-tetramethyl)hexadecyl-3-*O*-phytanyl-*sn*-glycerol (hydroxyarchaeol). Those structures were identified on the basis of chemical analysis, fast atom bombardment-mass spectrometry, gas-liquid chromatography-mass spectrometry and ^{1}H- and ^{13}C-NMR spectrometry. The latter was a new core lipid which was different from hydroxyarchaeol of *Methanothrix concilii*. The hydroxyarchaeol core lipid comprised 60% of polar lipid in *M. barkeri*. The structures of core lipids are quite different from those previously reported by De Rosa et al. (Biochim. Biophys. Acta (1986) 875, 487–492) concerning *M. barkeri* lipids. The structures of two major polar lipids, both of which had hydroxyarchaeol as core portions, were elucidated. These lipids were 2-*O*-(3′-hydroxy)phytanyl-3-*O*-phytanyl-*sn*-glycero-1-phosphoserine (hydroxyarchaetidylserine) and 2-*O*-(3′-hydroxy)phytanyl-3-*O*-phytanyl-*sn*-glycero-1-phospho-*myo*-inositol (hydroxyarchaetidyl-*myo*-inositol). Archaetidylserine and archaetidylinositol, which had the usual archaeol core portion, were also present as minor polar lipids.

1. Introduction

One of the most distinctive features of archaebacteria is their ether lipids. The ether core lipids (alkyl glycerol ether portion of polar lipids) of methanogens comprise principally diphytanyl glycerol diether (archaeol) and dibiphytanyl diglycerol tetraether (caldarchaeol). Recent reports have indicated the occurrence of structural variation of these core lipids in some species of methanogens; for example, macrocyclic diether in *Methanococcus jannaschii* [1], hydroxyarchaeol in *Methanothrix concilii* which has a 3′-hydroxyphytanyl chain at the *sn*-3 position of the glycerol backbone [2], and glycerol monoether, tetritol diether and caldarchaeols with various numbers of cyclopentane rings in the C_{40} hydrocarbon chains in *Methanosarcina barkeri* [3]. *M. barkeri* is used for many biochemical studies as a methylotrophic methanogen as well as a hydrogenotrophic methanogen, *Methanobacterium thermoautotrophicum*. Because the thin-layer chromatographic (TLC) pattern of total lipid of *M. barkeri* was found to be unique [4], structure elucidation of polar lipids was undertaken. In the course of this study, a new core lipid was found and the core lipid composition was quite different from that reported by De Rosa et al. [3]. The core lipid of this bacterium was composed of archaeol and hydroxyarchaeol. The latter is a new core lipid which has a hydroxyphytanyl chain at the *sn*-2 position of the glycerol moiety and is therefore different from hydroxyarchaeol of *M. concilii*. Two major polar lipids were structurally elucidated. These were hydroxyarchaetidylserine and hydroxyarchaetidyl-*myo*-inositol, both of which are new polar lipids having the hydroxyarchaeol as a core lipid. Archaetidylserine and archaetidyl-*myo*-inositol were also found as minor lipids. This report describes the membrane polar lipid composition and structure determinations of the new polar

Abbreviations: TLC, thin-layer chromatography; FAB-MS, fast atom bombardment-mass spectrometry.

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lipids which have the new hydroxyarchaeol core. The nomenclature for archaebacterial lipids proposed by us [5] is used in this text.

Materials and Methods

Growth of organisms

M. barkeri MS (DSM 800) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) and grown in a 1 l glass bottle containing 400 ml of medium No. 120 described in the DSM catalog 1989 containing 1% (v/v) methanol at 37°C under an atmosphere of $H_2 + CO_2$ (4 : 1) without shaking. *M. thermoautotrophicum* ΔH (DSM 1053) was grown as previously described [6]. *Sulfolobus solfataricus* strain P1 (DSM 1616) was obtained from DSM and grown in medium No. 182 (the DSM catalog 1989) at 70°C without shaking. The cells were harvested by centrifugation.

Extraction and purification of lipids

Total lipid of *M. barkeri* or *M. thermoautotrophicum* was extracted with the trichloroacetic acid-acidified solvent by the method of Bligh and Dyer [7] modified by Nishihara and Koga [6]. The usual Bligh and Dyer method was used for extraction of lipid from *S. solfataricus* and for some extraction experiments of *M. barkeri*. The total lipid (30–40 mg) of *M. barkeri* was fractionated on a column (1.0 × 10 cm) of DEAE-cellulose by elution with 200 ml each of chloroform (nonpolar lipids), and methanol containing 0.25 mol/l ammonium acetate (polar lipids) as described previously [8]. The polar lipid in the latter fraction was recovered by the Bligh and Dyer partition. Total lipid extracted by the acid Bligh and Dyer method from the cells of *Methanothrix soehngenii* (strain Opfikon, DSM 2139), which belongs to the same species as *M. concilii* [9], was a gift from Hiroyoshi Miyahara (TOTO, Kitakyushu, Japan). Lipid composition of *M. soehngenii* was almost the same as that of *M. concilii* (unpublished data). Purification of each polar lipid and core lipid was carried out by TLC with solvents B and C, respectively (see below).

Chromatography

TLC was carried out on a silica gel 60 plates (Merck Art 5721) with the following solvents: solvent A, chloroform/methanol/conc. aqueous ammonia (65 : 35 : 8, v/v); solvent B, chloroform/methanol/acetic acid/water (85 : 30 : 15 : 5, v/v); solvent C, light petroleum/ethyl ether/acetic acid (50 : 50 : 1, v/v). Solvents A and B were used for two-dimensional TLC for polar lipids in the first and second dimensions, respectively. Spots on silica gel plates were visualized by using iodine vapor (all lipids), ninhydrin (amino groups), acid molybdate (phospholipids), α-naphthol (glycolipids), periodate-Schiff reagent (vicinal hydroxyl groups), and 30% H_2SO_4 with subsequent charring at 175°C (all organic compounds) as described [6]. Cellulose TLC was used for analysis of water-soluble glycerophosphoesters prepared from polar lipids by BCl_3 treatment [10]. The cellulose TLC plate (Merck Art 5716) was developed with solvent D, phenol/water (100 : 38, v/v), and the spots on the plate were detected with ninhydrin and salicylsulfonic acid/$FeCl_3$ reagent for phosphoric esters [11].

GLC was performed on a Shimadzu GC9A gas chromatograph. Hydrocarbons and acetylated core lipids, were analyzed on a column (100 cm) packed with 2% Dexsil 300GC on Chromosorb W at a temperature increasing from 120 to 340°C at a rate of 20 C°/min. Hexacosane was used as an internal standard for the relative retention time of a sample. Acetylated inositol and sugar derivatives were chromatographed on a 200-cm column packed with 2% OV-225 on Uniport HP isothermally at 210°C. Trimethylsylilated inositol was analyzed under the same conditions except the temperature used at 175°C.

Analytical methods and chemical procedures

Phosphorus and sugar contents were measured by the methods of Bartlett [12] and Dubois et al. [13], respectively. Three methods were used to prepare core lipids. Those were mild acid methanolysis (0.18% HCl-methanol at 50°C for 24 h) [2], strong acid methanolysis (5% HCl-methanol at 100°C for 3 h) [6], and acetolysis [6] and subsequent strong acid methanolysis. Acetylation of archaeol, inositol, and sugar derivatives were carried out with a mixture of pyridine and acetic anhydride (1 : 1, v/v) at 100°C for 3 h, while hydroxyarchaeol was acetylated at room temperature overnight with the same reagent. Hydrocarbon chains were prepared from polar lipids by HI degradation followed by $LiAlH_4$ reduction as previously reported [8]. BCl_3 treatment [10], HF degradation [14], and preparation of sugar derivatives [5] were performed as previously described.

Physical measurements

Optical rotations were measured as a chloroform solution at 589 nm with a high-sensitivity polarimeter (PM-201, Otsuka Electronics, Japan). A mass spectrometer JMS DX-300 (Japan Electron Optics Laboratory, Japan) was used for GC-MS and fast atom bombardment-mass spectrometry (FAB-MS). A column (1 m) of 2% OV-1 was used for GC-MS at a temperature increasing from 120 to 320°C at a rate of 20 C°/min. FAB-MS spectra were obtained in a positive ion mode with a matrix of thioglycerol + NaI (core lipids) or *m*-nitrobenzyl alcohol (polar lipids). ^{13}C-NMR and ^{1}H-NMR were measured at 67.80 MHz with proton decoupling and at 270 MHz, respectively, by using a

JMN-GX-270 spectrometer (Japan Electron Optics Laboratory, Japan). The lipids were dissolved in chloroform-d_1 or dimethylsulfoxide-d_6 for NMR measurements.

Materials

Authentic samples of phytane, biphytane, C_{40} hydrocarbons with 1, 2 or 3 cyclopentane rings, archaeol and caldarchaeol were prepared from total lipid of *M. thermoautotrophicum* or *S. solfataricus*. Authentic lipids of archaetidylserine and archaetidyl-*myo*-inositol were purified from the total lipids of *M. thermoautotrophicum*. Chloroform-d_1 and dimethylsulfoxide-d_6 were purchased from E. Merck (Darmstadt, Germany).

Results

Lipid content and lipid composition

The total lipid extracted from the cells of *M. barkeri* by the acid Bligh and Dyer method accounted for 5.4% of the dry cell. The ratio of non-polar lipids to polar lipids was determined gravimetrically 13 : 87 in the total lipid. Phosphorus and sugar contents in the total lipid were 42.1 μmol and 3.7 μmol per g dry cell, respectively. The usual Bligh and Dyer extraction method was proved unsatisfactory because the extract accounted for only 2.9% of dry cell. Therefore, the acid extraction method was used throughout the experiments. The sugar moiety was liberated from the total lipid by strong acid methanolysis and identified by GLC as the forms of aiditol acetate, 1-methyl sugar acetate and acetylated sugar. Glucose was found as a sole sugar moiety in the lipids.

Fig. 1 shows a two-dimensional TLC chromatogram of the total lipid. At least 14 spots of polar lipids were detected by acid charring. The lipids were tentatively designated as shown in Fig. 1. They were classified on the basis of specific staining responses into three groups, phospholipids (six spots, designated as PL1-PL6), aminophospholipid (seven spots, designated as PNL1-PNL7), and glycolipid (one spot, designated as GL1). Table I shows the composition of these polar lipids. The predominant polar lipids (more than 10 mol%) were PL2, PL6, PNL4 and PNL6.

Structures of core lipids

GLC showed only one hydrocarbon chain in the polar lipid fraction which had a relative retention time (0.495) identical to that of authentic phytane. Other peaks such as C_{40} hydrocarbons with or without cyclopentane rings, which had relative retention times of 1.406–1.583, were not detected even when phytane was measured at a level 600 times higher than the lowest detection limit. This result is not incompatible with the presence of a hydroxyphytanyl chain in the polar lipids shown below, because the procedure for hydrocarbon

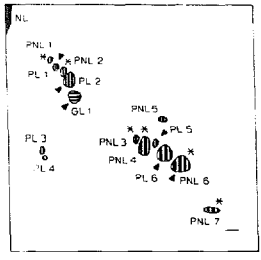

Fig. 1. Two-dimensional thin-layer chromatogram of total lipid of *M. barkeri*. PL, phospholipid; PNL, aminophospholipid; GL, glycolipid; NL, neutral lipid. Solvents A and B (see Materials and Methods) were used in the first (vertical) and second (horizontal) directions, respectively.

preparation would yield unsaturated derivatives of phytane from the hydroxyphytanyl chain by dehydration and the unsaturated derivatives could not be distinguished from saturated phytane under the conditions used.

Core lipids prepared by mild acid methanolysis consisted of two lipids, that is, lipid 1 and lipid 2 (R_F 0.58

TABLE I

Polar lipid composition of M. barkeri

Lipid	Mol% [a]
Phospholipid	
PL1	1
PL2	15
PL3	2
PL4	1
PL5	4
PL6	20
Aminophospholipid	
PNL1	1
PNL2	4
PNL3	2
PNL4	17
PNL5	1
PNL6	24
PNL7	2
Glycolipid	
GL1	6

[a] Mol% was calculated assuming that each lipid contained 1 phosphate moiety or 2 glucose moiety.

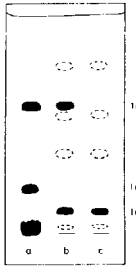

Fig. 2. Thin-layer chromatogram showing products of: (a) mild acid methanolysis of total polar lipids; (b) strong acid methanolysis of total polar lipid; (c) strong acid methanolysis of isolated lipid 2. Chromatography was developed with solvent C (see Materials and Methods). Black spots and encircled spots mean major lipids and minor lipids, respectively.

and R_F 0.19, respectively, Fig. 2, lane a). Because this treatment is not satisfactory for the complete cleavage of phospholipids, a significant fraction of polar lipids retained at the origin of the chromatogram, and therefore acetolysis and subsequent methanolysis were also attempted. In this case (Fig. 2, lane b), polar head groups were completely removed and lipid 2 remained no longer intact and was converted to lipid 3 (R_F 0.10), while lipid 1 was given as in mild methanolysis. No detectable amount of caldarchaeol with or without cyclopentane rings (R_F = 0.30–0.35 or 0.36) was detected in either case. Purified lipid 2 was completely degraded to lipid 3 as a major product upon strong acid methanolysis (Fig. 2, lane c). It is, therefore, concluded that the core lipids of this bacterium are composed of two species, lipid 1 and lipid 2. The latter is acid labile and lipid 3 is a degradation product of lipid 2. For complete understanding of core lipid structures of this organism, lipid 1, 2 and 3 were identified as described below.

Lipid 1 was identified as archaeol (2,3-di-*O*-phytanyl-*sn*-glycerol) on the basis of analyses with TLC, GLC, FAB-MS, GC-MS, ^{1}H- and ^{13}C-NMR (data not shown) and comparison of specific optical rotation ($[\alpha]_D$ = +8.84°) with standard archaeol.

FAB-MS of lipid 2 showed a molecular ion peak of m/z 691 ($[M + Na]^+$) as the only peak in a mass range above 400, corresponding to the molecular weight (668) of hydroxyarchaeol. A spectrum of GC-MS of its acetylated derivative showed two significant characteristic peaks of m/z 397 ($M - OC_{20}H_{40}OCOCH_3$) and m/z 355 ($OC_{20}H_{40}OCOCH_3$), the sum of which (752) was corresponding to the molecular weight of the diacetylhydroxyarchaeol. The specific optical rotation $[\alpha]_D$ was +5.65°, which was quite similar to but slightly different from that ($[\alpha]_D$ = +5.95°, Ref. 2) of hydroxyarchaeol of *M. concilii*. The ^{1}H-NMR spectrum of lipid 2 clearly showed the presence of two hydroxyl group in the molecule, one of which was attributable to the hydroxyl group in the aliphatic region and the other to that of glycerol moiety. The mass spectral data was not shown here because it was the same as that of *M. concilii* [2].

^{13}C-NMR spectra of archaeol (lipid 1) and hydroxyarchaeol (lipid 2) were compared in order to establish the structure of lipid 2. Signals were assigned according to the data of *M. concilii* [2]. Table II shows the chemical shifts of the carbon atoms from C1′ to C5′ and C17′ of phytanyl chains of the core lipids. (see Fig. 3 for numbering of carbon atoms of the phytanyl chains). The chemical shifts of the other carbon atoms of the molecule were similar in archaeol and hydroxyarchaeol and are not shown here. The most significant difference between archaeol and hydroxyarchaeol was observed in the C3′ signal. In the spectrum of hydroxyarchaeol, one (on the *sn*-2 chain) of two methine signals found in archaeol was missing and a new peak appeared downfield at 72.47 ppm. The other one (on the *sn*-3 chain, 29.93 ppm) was not affected. This strong downfield shift of the C3′ signal, along with shifts of the other carbon atoms, were attributed to the presence of a hydroxyl group on the C3′ position of one of two phytanyl chains in the archaeol molecule. Of the two phytanyl chains (bound at the *sn*-2 and *sn*-3 positions of the glycerol moiety), the hydroxylated hydrocarbon chain was found to be linked at the *sn*-2 position, because the C2′ signal (39.97 ppm) of the *sn*-2 chain of *M. barkeri* hydroxyarchaeol showed a significant shift

TABLE II

^{13}C-NMR chemical shifts for phytanyl chains of archaeol and hydroxyarchaeol

Chemical shifts are given in ppm relative to tetramethylsilane as an internal standard. Carbon atoms were numbered as illustrated in Fig. 3.

Carbon number	Archaeol (lipid 1)	Hydroxyarchaeol (lipid 2)
1′	68.68 (68.67) [a] (*sn*-2)	67.49 (68.77) (*sn*-2)
	70.19 (70.19) (*sn*-3)	70.23 (68.90) (*sn*-3)
2′	37.13 (37.11) (*sn*-2)	39.97 (37.10) (*sn*-2)
	36.63 (36.62) (*sn*-3)	36.59 (39.78) (*sn*-3)
3′	29.90 (29.88)	72.47 (29.90) (*sn*-2)
	29.93 (29.92)	29.92 (72.37) (*sn*-3)
4′	37.33–37.53	43.12 (37.32–37.57) (*sn*-2)
	(37.32–37.53)	37.31–37.48 (42.92) (*sn*-3)
5′	24.39 (24.38)	21.49 (24.39) (*sn*-2)
		24.39 (21.51) (*sn*-3)
17′	19.72 (19.70–19.77)	26.66 (19.67–19.77) (*sn*-2)
	19.78	19.76–19.71(26.67) (*sn*-3)

[a] Values in the parenthesis are data of *M. concilii* lipids [2].

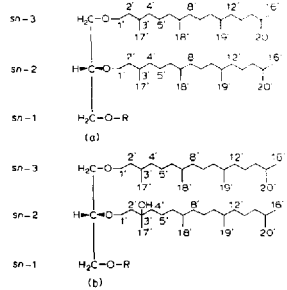

Fig. 3. Proposed structures of lipids of *M. barkeri*. Carbon atoms of methyl groups attached to 3', 7', 11' and 15'-positions of hexadecyl chain of phytanyl residue were numbered 17', 18', 19' and 20', respectively. (a) R = H (archaeol); R = phosphoserine (archaetidylserine, PNL3); R = phospho-*myo*-inositol (archaetidylinositol, PL5). (b) R = H (hydroxyarchaeol); R = phosphoserine (hydroxyarchaetidylserine, PNL4); R = phospho-*myo*-inositol (hydroxyarchaetidylinositol, PL6).

compared to that of archaeol (37.13 ppm), while that of the *sn*-3 chain was not. In the case of *M. concilii*, the C2' signal of the *sn*-3 chain was shifted and that of the *sn*-2 chain was not. The C1' signal of the *sn*-2 chain in *M. barkeri* also showed a smaller shift. These results indicated that the structure of lipid 2 is 2-*O*-(3'-hydroxy)phytanyl-3-*O*-phytanyl-*sn*-glycerol, and it was further confirmed by the determination of the structure of lipid 3, the degradation product of hydroxyarchaeol (lipid 2).

Lipid 3 showed a slightly higher R_F value (0.10) on TLC compared to 2-*O*-phytanyl-*sn*-glycerol ($R_F = 0.09$) from *M. soehngenii*. Lipid 3 showed positive reaction to periodate-Schiff reagent on a TLC plate while 2-*O*-phytanyl-*sn*-glycerol from *M. soehngenii* was negative. A diacetylated derivative of lipid 3 showed a similar but slightly different relative retention time (1.082) as that (1.091) of acetylated 2-*O*-phytanyl-*sn*-glycerol on GLC. The molecular weight of lipid 3 (372) was confirmed by FAB-MS (m/z 373, $[M+1]^+$; m/z 395, $[M+Na]^+$). In GC-MS of the acetylated derivative of lipid 3, peaks consistent to the structure of *O*-phytanyl-*sn*-glycerol were observed. A peak of m/z 311 ($H_2COC_{20}H_{41}$) which is consistent to the structure of α-*O*-phytanyl glycerol was present as the highest peak in a mass range over m/z 300. Chemical shifts of ^{13}C-NMR of carbon atoms in glycerol moiety and at the 1'-position of phytanyl chain also proved that it was an α-isomer of *O*-phytanyl glycerol (data not shown). The specific optical rotation $[\alpha]_D$ of lipid 3 was $-0.96°$, which was different from $[\alpha]_D$ $+2.69°$ of 2-*O*-phytanyl-*sn*-glycerol [2] but identical to $[\alpha]_D$ $-0.94°$ of 3-*O*-phytanyl-*sn*-glycerol [15]. Thus lipid 3 is concluded to be 3-*O*-phytanyl-*sn*-glycerol and confirmed the structure of lipid 2 as shown in Fig. 3b (R = H).

The molecular ratio of archaeol to hydroxyarchaeol was determined most likely after the complete removal of phosphoester groups and glycosidic groups by acetolysis and methanolysis. GLC was carried out after acetylation of the cores. The ratio measured 4 : 6.

Structures of PNL4 and PNL3

PNL 4 was positive to ninhydrin and acid-molybdate and negative to a-naphthol and periodate-Schiff reagents. The R_F values of 0.37 and 0.38 of this lipid on TLC with solvent A and B, respectively , were close but slightly lower to those (0.41 and 0.42) of the authentic archaetidylserine from *M. thermoautotrophicum*. A gas chromatogram of the hydrocarbon prepared by HI degradation followed by $LiAlH_4$ reduction showed a single peak which coincided with that of phytane. Acetolysis and subsequent strong acid methanolysis completely removed the polar group to yield 3-*O*-phytanyl-*sn*-glycerol. This result shows that the core portion of this lipid is hydroxyarchaeol. Although mild acid methanolysis did not cleave the polar head group, intact hydroxyarchaeol was obtained by HF reaction, and at the same time, serine (and ammonia) were detected by an amino acid analyzer as water-soluble products of HF degradation. The water-soluble product of BCl_3 treatment of this lipid cochromatographed with the authentic glycerophosphoserine on cellulose TLC. The polar head group of PNL4 was, therefore, identified as phosphoserine. The positive FAB-MS of PNL4 gave a molecular ion peak of m/z 858 $[M+Na]^+$ as the highest peak in the mass range above m/z 550, which is consistent with the molecular weight (835) of hydroxyarchaetidylserine. Finally, the structure of PNL4 was identified as hydroxyarchaetidylserine (2-*O*-(3'-hydroxy)phytanyl-3-*O*-phytanyl-*sn*-glycero-1-phosphoserine. Fig. 3b, R = phosphoserine).

PNL3, which is a minor lipid and located close to PNL4 on two-dimensional TLC, had the same R_F values as those of authentic archaetidylserine and was identified as archaetidylserine (Fig. 3a, R = phosphoserine) on the basis of chemical degradation analysis and FAB-MS. The data are not shown here because they were essentially the same as those for archaetidylserine of *Methanobrevibacter arboliphilicus* in the previous report [14].

Structures of PL6 and PL5

PL6 was positive to acid molybdate and periodate-Schiff but negative to α-naphthol and ninhydrin reagents. The R_F values of this lipid on TLC with solvent A and B were 0.34 and 0.30, respectively. These values were similar but slightly lower than those (0.39 and

0.34) of the authentic archaetidyl-*myo*-inositol. A single peak corresponding phytane was detected in GLC of hydrocarbon prepared from this lipid. Mild acid methanolysis completely cleaved the polar head group and yielded hydroxyarchaeol. Strong acid methanolysis of the intact lipid produced 3-*O*-phytanyl-*sn*-glycerol. The water soluble product of the strong acid methanolysis was further hydrolyzed by 6 mol/l HCl for 6 h. *myo*-Inositol was found in the hydrolysate by GLC after acetylation or trimethylsilylation. A water-soluble product of BCl_3 treatment of this lipid cochromatographed with glycerophospho-*myo*-inositol prepared from the authentic archaetidylinositol. These results show that PL6 has hydroxyarchaeol and phospho-*myo*-inositol which are bound each other through a phosphodiester linkage (molecular weight = 910). The positive FAB-MS spectra obtained by using a matrix of thioglycerol, gave a molecular ion peak of m/z 933 ($[M + Na]^+$) as the highest peak in a mass range above m/z 530, confirming the structure of PL6 as hydroxyarchaetidylinositol (2-*O*-(3′-hydroxyl)phytanyl-3-*O*-phytanyl-*sn*-glycero-1-phospho-*myo*-inositol, Fig. 3b, R = phospho-*myo*-inositol).

PL5, a minor lipid which is located close to PL6 on TLC, was identified as archaetidyl-*myo*-inositol (Fig. 3a, R = phospho-*myo*-inositol) on the basis of chemical degradation analysis and FAB-MS. The data are not shown because they were essentially the same as those of archaetidyl-*myo*-inositol in *M. thermoautotrophicum* [8].

Discussion

It was shown that the core lipids of the membrane lipids of *M. barkeri* consisted of only two species, archaeol and hydroxyarchaeol. No other kind of core lipid was detected in the organism. The hydroxyarchaeol from *M. barkeri* resembles but is an isomer of another hydroxyarchaeol from *M. concilii*. Therefore, this is a new core lipid. The new hydroxyarchaeol core has a hydroxyl group at the C3′ of the phytanyl chain linked to the *sn*-2 position of glycerol in contrast with the hydroxyarchaeol of *M. concilii* which has a hydroxyl group on the *sn*-3 chain. Recently, Sprott et al. [16] have identified independently the same structure of hydroxyarchaeol from *M. barkeri* and *Methanosarcina mazei*.

It is unlikely that hydroxyarchaeol is an artifact which is formed by hydration of archaeol with unsaturated hydrocarbon chains during the acidic extraction, because hydroxyarchaetidylserine and hydroxyarchaetidyl-*myo*-inositol were also present as major polar lipids in the total lipids obtained by the usual (neutral) Bligh and Dyer extraction method (data not shown).

It was shown that hydroxyarchaeol (lipid 2) was labile to strong acid methanolysis and the treatment removed the hydroxyphytanyl chain at the *sn*-2 position of the glycerol moiety. This reaction is reasonably expected as follows. 3′-Hydroxyphytanyl chain is a tertiary alcohol which tends to be converted to 2′-enyl group (allyl group) by dehydration at 2′ and 3′ positions of the hydroxyphytanyl chain. The resultant allyl ether is easily hydrolyzed in the presence of H^+. Acid lability of the allyl ether could be greatly higher than saturated ethers. Thus, it can be expected that hydroxyarchaeol is converted to monoalkyl glycerol by the strong acid methanolysis.

De Rosa et al. [3] reported that structures of core lipids of the same bacterial strain consisted of archaeols (C_{20}-C_{20} type and C_{20}-C_{25} type), monoalkylglycerol ether, tetritol (butane tetrol) diether and caldarchaeol with various numbers of cyclopentane rings. A part of the discrepancy may be explained by the fact that they employed strong acid methanolysis in preparation of core lipids, which yields *O*-phytanyl glycerol and a small amount of degradation products (Fig. 2, and Ref. 2). Those may easily lead to confusion in the identification of core lipids. The contradiction of the presence and absence of caldarchaeol core lipid is, however, not explained. The evidence from our experiments was all against the presence of caldarchaeol core lipids. Although De Rosa et al. [3] described that caldarchaeol comprised 17% of total core lipids, C_{40} hydrocarbon, if any, was present at the level below 1/600 of phytane in our measurement.

Two new polar lipids, hydroxyarchaetidylserine and hydroxyarchaetidyl-*myo*-inositol were found in this bacterium as major lipids along with minor lipids of archaetidylserine and archaetidyl-*myo*-inositol. The formation of glycerophosphoserine and glycerophosphoinositol by BCl_3 treatment of PNL4 and PL6 excluded the possibility that phosphoserine and phosphoinositol are bound to the hydroxyl group of the hydroxyphytanyl chain of each lipid. The polar head group of hydroxyarchaetidylserine, along with that of archaetidylserine, was not cleaved by acid methanolysis while hydroxyarchaetidyl-*myo*-inositol and archaetidyl-*myo*-inositol were hydrolyzed to yield core lipids. This is probably due to the fact that the serine lipids lack a free hydroxyl group on a carbon atom (for example, the position 2 of *myo*-inositol) adjacent to the carbon atom which is linked to the phosphate group. The free hydroxyl group is essential for hydrolysis of phosphodiester bond, being involved in the formation of a cyclic phosphodiester compound as an intermediate. Each polar lipid containing hydroxyarchaeol migrated to a slightly lower position on TLC compared to the corresponding phospholipid with the same polar head group and archaeol core. The TLC chromatogram (Fig. 1) suggests the possibility that there are other kinds of paired polar lipids with and without a hydroxyl group in their hydrocarbon chains.

Polar lipids with a hydroxyarchaeol core is now known to be present in *M. concilii* [17,18] and *M. barkeri*, both of which belong to the family of *Methanosarcinaceae*. The structures of hydroxyarchaeol cores of these organisms were similar but different in the strict sense of the term, while the compositions of major polar lipids were greatly different. Archaetidyl-*myo*-inositol, gentiobiosyl hydroxyarchaeol and gentiobiosylarchaeol were the major lipids in *M. concilii* [17]. In *M. concilii*, hydroxyarchaeol core is contained mainly in major glycolipids but phospholipids containing hydroxyarchaeol are minor lipids. In the case of *M. barkeri*, hydroxyarchaetidyl-*myo*-inositol and hydroxyarchaetidylserine predominated. *M. concilii* does not have a detectable amount of hydroxyarchaetidylserine or archaetidylserine [17,18]. The other two major polar lipids in *M. barkeri*, PL2 and PNL6, are expected to be structurally different from the major polar lipids of *M. concilii* because of their different chromatographic behaviors. Eventually, polar lipid constituents could represent more properly the difference of the genera than core lipids. Therefore, it is important to elucidate complete structures of major polar lipids of *Methanosarcinaceae*. Structure elucidation of the other major polar lipids, PL2 and PNL6, is now in progress and will be published elsewhere.

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