J. Biochem. **101**, 1007–1015 (1987)

Structure Determination of a Quartet of Novel Tetraether Lipids from *Methanobacterium thermoautotrophicum*[1]

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Received for publication, September 29, 1986

The structures of three of the major polar lipids (PNL1a, GL1a, and PNGL1) of *Methanobacterium thermoautotrophicum* were elucidated. These lipids are derivatives of dibiphytanyl diglycerol tetraether (C_{40} tetraether; the proposed name is caldarchaeol). PNL1a is a C_{40} tetraether analog of phosphatidylethanolamine (proposed name: caldarchaetidylethanolamine). GL1a was identified as β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl C_{40} tetraether (diglucosyl caldarchaeol). PNGL1 has the polar head groups of both PNL1a and GL1a; one of the free hydroxyls of this tetraether is esterified with phosphoethanolamine while the other is linked to a glucosylglucose residue with the same structure as that of GL1a (proposed name: diglucosyl caldarchaetidylethanolamine). That is, PNL1a (aminophospholipid), GL1a (glycolipid), and PNGL1 (aminophosphoglycolipid) form structurally a quartet of lipids with the bare caldarchaeol. We propose a new systematic nomenclature of archaebacterial polar lipids in the "DISCUSSION," because the alternative names are too lengthy and laboratory designations of these lipids are not at all systematic. This nomenclature starts with giving the names archaeol and caldarchaeol to dialkyl diether of glycerol or other polyol and tetraether of glycerol or other polyol and alkyl alcohols, respectively, because these lipids are specific to archaebacteria. Phospholipids with a phosphodiester bond were named as derivatives of archaetidic acid or caldarchaetidic acid (phosphomonoesters of archaeol and caldarchaeol) by analogy with phosphatidic acid.

Although the structures of polar lipids of *Methanospirillum hungatei* (*1*) and *Methanobrevibacter arboriphilus* A2 (*2*) have been elucidated, those from *Methanobacterium thermoautotrophicum* have not yet been studied at all. A wide variety of biochemical knowledge of *M. thermoautotrophicum* ΔH is being accumulated, since this is a representative strain of methanogen (*3*), and data on the lipid biochemistry of methanogens should be integrated into the bulk of information on *M.*

[1] This study was supported in part by Grants-in-Aid for Scientific Research (Nos. 59560094 and 61760088) from the Ministry of Education, Science and Culture of Japan, and a grant from the Agricultural Chemistry Foundation. Abbreviations: C_{20} diether, diphytanyl glycerol diether; C_{40} tetraether, dibiphytanyl diglycerol tetraether; FAB, fast atom bombardment.

thermoautotrophicum for effective discussions concerning methanogen biochemistry. The extraction conditions and composition of lipids from *M. thermoautotrophicum* were established in the previous paper (*3*). The Bligh and Dyer solvent (*4*) acidified with trichloroacetic acid was found to be the most convenient and effective for the extraction of lipids from the cells. The nonpolar core groups of the lipids were mainly composed of dibiphytanyl diglycerol tetraether (C_{40} tetraether, proposed name caldarchaeol; see "DISCUSSION"). We found 6 aminophospholipids and 3 aminophosphoglycolipids as well as 10 phospholipids, 2 phosphoglycolipids, and 2 glycolipids without an amino group. The designations of these lipids were given (*3*). Among 23 polar lipids, an aminophospholipid, a glycolipid, and an aminophosphoglycolipid were structurally elucidated, as described in the present paper. The aminophospholipid (designated as PNL1a; proposed name, caldarchaetidylethanolamine) has a phosphoethanolamine residue and the glycolipid (designated as GL1a; diglucosyl caldarchaeol) contains a glucosylglucose residue. The third lipid, designated as PNGL1 (diglucosyl caldarchaetidylethanolamine), possesses both phosphoethanolamine and glucosylglucose residues. Thus, these lipids constitute a quartet of tetraether lipids in company with the bare C_{40} tetraether (caldarchaeol) which is the core lipid of the above three lipids. A systematic nomenclature of polar ether lipids from archaebacteria is proposed.

MATERIALS AND METHODS

Growth of the Bacterium and Extraction of Lipids—Growth conditions of *M. thermoautotrophicum* ΔH (DSM1053) and the method of extraction of lipids by using the acidified solvent with trichloroacetic acid were described previously (*3*).

Thin-Layer Chromatography—TLC was carried out on Silica Gel 60 plates (Merck) using the following solvents (composition in volume ratios): solvent A, chloroform–methanol–7 M aqueous ammonia (60 : 35 : 8); solvent B, chloroform–acetone–methanol–acetic acid–water (200 : 150 : 100 : 15 : 10); solvent C, light petroleum–diethyl ether–acetic acid (50 : 50 : 1); solvent D, light petroleum–diethyl ether–methanol–acetic acid (150 : 150 : 10 : 3). Lipid spots were detected as described in the previous paper (*3*). Water-soluble dealkylated products were chromatographed on cellulose TLC plates (Avicel SF plate, Asahi Kasei Co., Inc.) with solvent E, phenol–water (100 : 38). Spots on the cellulose plate were detected with salicylsulfonic acid/$FeCl_3$ reagent for phosphate esters (*5*) and ninhydrin.

Purification of Lipids—Total lipids (400–500 mg) were fractionated on a column (2.5 × 40 cm) of DEAE-cellulose (Brown, acetate form), as previously reported (*2*). GL1a, PNL1a, and PNGL1 were eluted with 2,000 ml each of the following solvent mixtures; chloroform–methanol (9 : 1), chloroform–methanol (8 : 2) and chloroform–methanol (1 : 1), respectively. Because GL1b, PNL1b, and PNGL3 contaminated the fractions of GL1a, PNL1a, and PNGL1, respectively, these fractions were further purified by TLC with solvent B. The R_f values of GL1a, PNL1a, and PNGL1 in this solvent were 0.69, 0.37, and 0 19, respectively.

Gas-Liquid Chromatography—GLC was performed by the use of a Shimadzu GC 6AM gas-liquid chromatograph equipped with flame ionization detectors. Trimethylsilyl derivatives of sugars were analyzed on a 2.0 m glass column packed with 3% SE-30 on Uniport HP (80–100 mesh) at a temperature increasing from 180 to 250°C at a rate of 2°C/min. Alditol acetates of sugars and acetylated methylglycosides were chromatographed on a 3 m glass column packed with 2% OV-225 on Uniport HP (60–80 mesh) at a temperature increasing from 160 to 220°C at a rate of 0.5°C/min. In the case of partially methylated alditol acetates of sugars, a temperature increasing from 130 to 190°C was used. Alkyl chlorides were analyzed on a 2% OV-11 on Chromosorb W AW-DMCS (80–100 mesh) column (2 m) at a temperature increasing from 100 to 330°C at a rate of 2°C/min.

Analytical Methods and Degradative Procedures—Determination of phosphorus and sugar, acid methanolysis, and acetolysis were carried out as described previously (*3*). Mild alkaline methanolysis was performed as described by Kates (*6*). The glycerol ether bond was cleaved with BCl_3 according to the method of Kates *et al.* (*7*), except that the incubation time was reduced to 4 h. After removal of borate as the volatile methyl ester by repeated addition of methanol and

evaporation, the products were taken by Bligh and Dyer partitioning between chloroform and aqueous methanol. The chloroform-soluble products (alkyl chlorides) were analyzed by GLC. A portion of the aqueous methanol-soluble product (glycerol or glycerophosphate esters) was chromatographed on a thin-layer cellulose plate with solvent E. Another portion of the fraction was subjected to hydrolysis with 2 M HCl at 100°C for 6 h and then glycerol was determined by the method of Kates (*8*). The phosphodiester bond was hydrolyzed with hydrofluoric acid as described (*2*). Amino compounds in the aqueous fraction of the hydrolyzate were identified and determined with an amino acid analyzer (model 835, Hitachi, Japan).

Sugar Analysis—Permethylation of glycolipids and phosphoglycolipids and the conversion of free and partially methylated sugars to alditol acetate were performed by the methods of Yang and Hakomori (*9*). Methylglycosides obtained by acid methanolysis of glycolipids or phosphoglycolipids were acetylated with pyridine and acetic anhydride (1 : 1, v/v) at 100°C for 1 h. Trimethylsilyl derivatives of sugars were prepared by treatment in *N*-trimethylsilylimidazole for 30 min at room temperature. For the measurement of optical rotation of sugars, the free sugars were purified as acetylated sugars by TLC with solvent D. Free sugars were regenerated from the purified acetylated sugars by acid methanolysis followed by 2 M HCl hydrolysis at 100°C for 2 h. Anomeric configurations of sugar moieties of glycolipids and phosphoglycolipids were determined by oxidation with CrO_3. Samples (400–600 μg hexose) of GL1a and PNGL1 were acetylated and then oxidized with CrO_3 by the method of Laine and Renkonen (*10*) with inositol (500 μg) as an internal standard and then hydrolyzed, and the sugars were converted to alditol acetates and analyzed by GLC.

Physical Measurements—Infrared spectra of the lipid were recorded as thin films using a Shimadzu IR spectrometer (IR450S). Optical rotations were measured at 25°C at 589 nm with a Union Giken high-sensitivity polarimeter PM-71 (Union Scientific Eng. Co., Ltd., Japan). Fast atom bombardment (FAB)-mass spectra were obtained with a mass spectrometer (JMS DX-300, Japan Electron Optics Laboratory, Japan), with glycerol plus 15-crown-5 as a matrix in a negative ion mode (*2*) or with a mass spectrometer (JMS D-300, Japan Electron Optics Laboratory) in a positive ion mode. The matrices were thioglycerol + NaI for GL1a and thioglycerol + 15-crown-5 for PNL1a and PNGL1.

Materials—Authentic samples of C_{20} diether and C_{40} tetraether were prepared from cells of *M. hungatei* GP1 (DSM1101) as described previously (*2*). Phytanyl chloride and biphytanyl dichloride were prepared as described above from the total lipid of *M. hungatei* GP1. Phosphatidylethanolamine (bovine brain) was the product of Avanti Polar Lipids, Inc. Phosphoethanolamine was purchased from Nakarai Chemicals Inc., Japan. BCl_3 (99.999% pure) was from Wako Pure Chemicals Inc., Japan. Glycerophosphoethanolamine was prepared from standard diacyl phosphatidylethanolamine by mild alkaline methanolysis. Sugar derivatives for use as standards in GLC analysis were prepared from commercially available glucose, galactose, mannose, lactose, sophorose, laminarin, maltose, and isomaltose.

RESULTS

D-Glucose as the Sole Sugar in the Total Lipid—Sugar moieties of glycolipids and phosphoglycolipids were completely liberated by acid methanolysis as previously reported (*3*). The released methylglycosides from the total lipid were converted to trimethylsilyl derivatives, alditol acetates, and acetylated methylglycosides and analyzed by GLC. Only derivatives of glucose were found on the chromatograms. The free sugar released and purified from the total lipid had an optical rotation of $[\alpha]_D$ +53.8° or M_D +96.8°, showing that the glucose in the total lipid was D-glucose.

Stereochemical Configurations of C_{20} Diether and C_{40} Tetraether Residues—C_{20} diether and C_{40} tetraether were purified from the total lipid after the polar head groups had been completely cleaved by acetolysis followed by acid methanolysis (*3*). The optical rotation of C_{20} diether was $[\alpha]_D$ +8.43° or M_D +55.0°, which was identical with that of 2,3-di-*O*-phytanyl *sn*-glycerol (M_D +55°) from *M. hungatei* (*1*). The M_D of *sn*-1,2-isomer was −40° (*1*). C_{40} tetraether showed a rotation of $[\alpha]_D$ +8.54° or M_D +110°, in agreement with that ($[\alpha]_D$ +8.7° or M_D +113°) from *M. hungatei* (*1*) in which the biphytanyl groups are linked to the

sn-2 and *sn*-3-positions of both glycerol residues. The M_D values of the *sn*-1,2-*sn*-2,3-isomer and *sn*-1,2-*sn*-1,2-isomer were $+15°$ and $-80°$, respectively (*1*). That is, *M. thermoautotrophicum* glycerol ether lipids have the same stereochemical configurations as those usually found in archaebacteria.

PNL1a—This lipid was positive to ninhydrin and molybdate reagents and negative to α-naphthol and periodate-Schiff reagents. The IR spectrum of the free acid form of PNL1a (Fig. 1a) showed absorptions corresponding to groups of methyl $-CH_3$ and methylene $-CH_2-$ (2,850–2,960, 1,465 and 1,380 cm^{-1}), ether C–O–C (1,100 cm^{-1}), phosphate P=O, $P-O^-$, and P–O–C (1,220, 1,090, and 1,055 cm^{-1}), amino NH_2 (3,390, 1,640, and 1,560 cm^{-1}) and primary alcohol C–OH (1,035 cm^{-1}). The R_f values of 0.76 and 0.37 of this lipid on TLC with solvents A and B, respectively, were close to those (0.73 and 0.36) of authentic phosphatidylethanolamine. Acid methanolysis caused no change in PNL1a while the authentic phosphatidylethanolamine was completely degraded. An ether bond resists acid methanolysis, while an ester bond of diacyl phospholipids can be readily methanolyzed to produce free hydroxyl groups, which serve as an essential participant in the formation of cyclic phosphate as the intermediate of hydrolysis of phosphodiester. Acetolysis of PNL1a cleaved the phosphodiester bond completely. To remove acetyl groups added during acetolysis, the acetolysis product was methanolyzed with mild alkali or acid, yielding a single compound which was identified as C_{40} tetraether by TLC with solvent C. BCl_3 treatment of the intact lipid produced biphytanyl dichloride. That is, PNL1a has C_{40} tetraether as a core lipid. The ratio of phosphate and glycerol was 1 : 2.02, indicating that PNL1a has one phosphate group in the molecule.

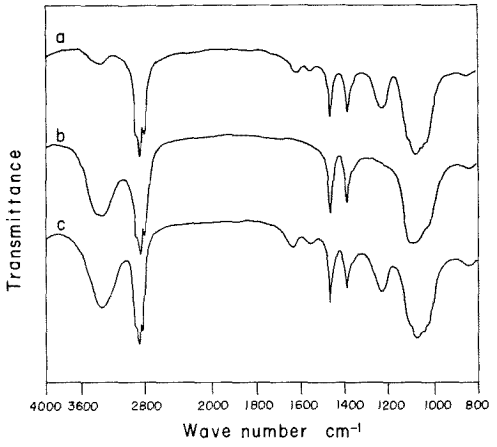


Fig. 1. IR spectra of PNL1a, GL1a, and PNGL1 recorded on thin films. a, PNL1a; b, GL1a; c, PNGL1.

It was found that BCl_3 cleavage of ether bonds was applicable to determine the structure of the polar head group because BCl_3 treatment hardly affected phosphodiester (Table I). After BCl_3 treatment of PNL1a, no phosphorus remained in

TABLE I. Stoichiometric conversion of PNL1a and PNGL1 by BCl_3 treatment. After treatment of the lipids with BCl_3 the chloroform-soluble and aqueous methanol-soluble fractions were separated. The aqueous methanol-soluble products were chromatographed on a cellulose TLC plate with solvent E. Two ninhydrin- and molybdate-positive spots were identified as glycerophosphoethanolamine and phosphoethanolamine. Phosphorus contents in these fractions were determined.

	Phosphorus			
	PNL1a		PNGL1	
	(nmol)	(%)	(nmol)	(%)
Original lipid	645	100	661	100
After BCl_3 treatment				
Glycerophosphoethanolamine	634	98.3	655	99.1
Phosphoethanolamine	6	0.9	4	0.6
P_i	0	0	0	0
$CHCl_3$-soluble fraction	0	0	0	0

the chloroform phase and no inorganic phosphorus was detected in the aqueous methanol phase. These results showed that the phosphate group was still bound to water-soluble organic compounds while the ether bonds were completely broken. TLC analysis of the aqueous methanol-soluble products showed the presence of one major organic phosphate-containing material accompanied with a minor spot, both of which were ninhydrin-positive. These compounds comigrated with authentic glycerophosphoethanolamine and phosphoethanolamine (R_f=0.59 and 0.37, respectively), and the former accounted for more than 98% of the phosphate in the original lipid (Table I). BCl_3 treatment of authentic phosphatidylethanolamine (diacyl form) gave the same recovery of aqueous methanol-soluble products, 97.5% of glycerophosphoethanolamine and 1.2% of phosphoethanolamine. The polar head group of PNL1a was, therefore, concluded to be phosphoethanolamine which was bound to the glycerol residue through a phosphodiester linkage. This was confirmed by HF hydrolysis of PNL1a, by which 70% of the original PNL1a was hydrolyzed, producing ethanolamine and NH_3 (72 and 33 mol% of the degraded PNL1a). The molecular weight of the C_{40} tetraether having phosphoethanolamine as a polar head should be 1,424.3. As expected, the FAB-mass spectra of PNL1a gave *m/z* 1,422 ($[M-2]^-$) in the negative ion mode and 1,425.4 ($[M+1]^+$), 1,424.5 ($[M]^+$) in the positive ion mode as the highest peaks in the mass range above *m/z* 1,000. It is concluded that PNL1a is a tetraether analog of phosphatidylethanolamine (proposed name, caldarchaetidylethanolamine; Fig. 2a).

GL1a—This lipid was a major glycolipid (16%, Ref. *3*) and gave positive responses to α-naphthol and periodate-Schiff reagents and negative responses to acid molybdate and ninhydrin on a TLC plate. The IR spectrum of GL1a (Fig. 1b) showed absorptions corresponding to groups of hydroxy OH (3,400 cm^{-1}), methyl $-CH_3$ and methylene $-CH_2-$ (2,850–2,960, 1,465, 1,380 cm^{-1}), ether C–O–C (1,100 cm^{-1}), and primary alcohol C–OH (1,035 cm^{-1}). No ester carbonyl (1,740 cm^{-1}) or sulfate absorption (1,265 cm^{-1}) was found. Acid methanolysis of GL1a yielded C_{40} tetraether as a chloroform-soluble product together with glucose in the aqueous methanol phase. The molar ratio of hexose (glucose) and glycerol was 1 : 0.98. This indicated that GL1a possesses two glucose moieties in one molecule because the core lipid C_{40} tetraether has two glycerol residues. These results suggested that GL1a is C_{40} tetraether having one glucose moiety at each free hydroxyl group of two glycerols or a glucosylglucose moiety at one side of the tetraether (molecular weight = 1,625.4). The positive ion FAB-mass spectrum of GL1a gave peaks of *m/z* 1,648.4 ($[M+Na]^+$) and 1,649.0 ($[M+Na+1]^+$) as the highest peaks found in the mass range above *m/z* 1,000 with a relative intensity of 1 : 0.852, confirming the structure of GL1a as diglucosyl C_{40} tetraether.

GLC analysis of the alditol acetates of the partially methylated glucoses derived from per-

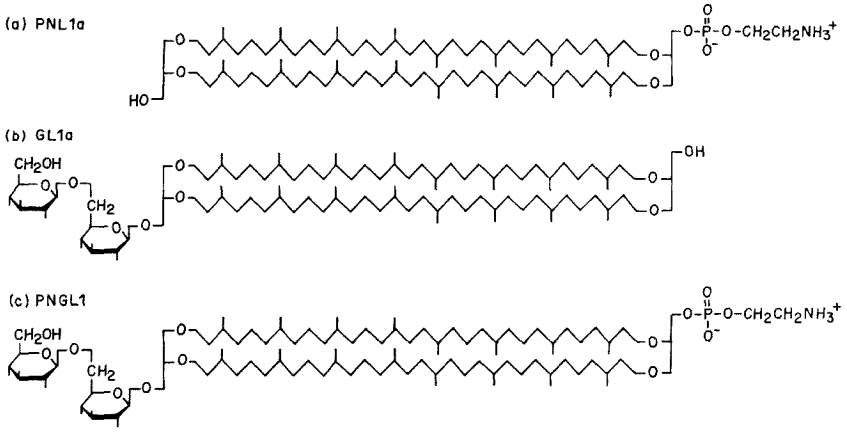
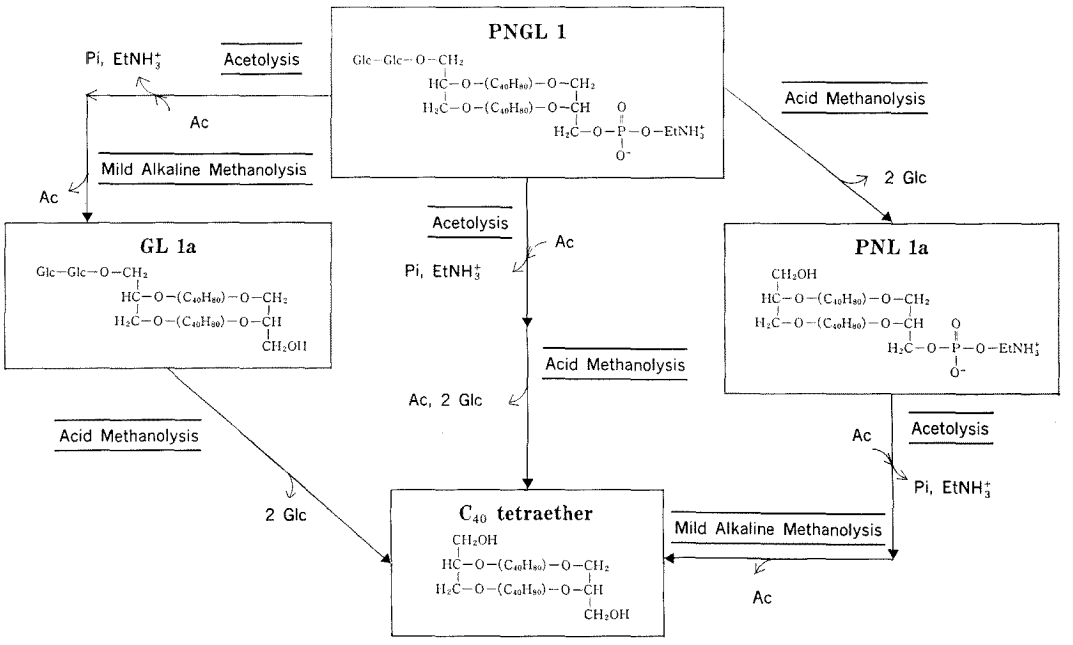


Fig. 2. Structures of PNL1a (a), GL1a (b), and PNGL1 (c).

methylated GL1a showed the presence of two peaks in an approximately equimolecular ratio. These two peaks (relative retention times of 1 and 1.609) were identified as 1,5-diacetyl 2,3,4,6-tetramethyl glucitol and 1,5,6-triacetyl 2,3,4-trimethyl glucitol on the basis of complete coincidence of the retention times with those of authentic partially methylated alditol acetates prepared from isomaltose. The presence of the triacetyl trimethyl derivative indicated that the two glucose residues formed glucopyranosyl-(1-6)-glucopyranose which was, in turn, linked to one hydroxyl group of C_{40} tetraether. To determine the anomeric configuration of the glycosides, CrO_3 oxidation was carried out since CrO_3 preferentially oxidizes hexosides with the β-configuration (*10*). CrO_3 oxidation of the acetylated GL1a completely destroyed the glucose in the lipid. Thus both glucosides were found to have β-configuration. Finally, the structure of GL1a was identified as β-glucopyranosyl-(1-6)-β-glucopyranosyl C_{40} tetraether (Fig. 2b; proposed name, gentiobiosyl caldarchaeol).

PNGL1—This lipid showed positive responses to ninhydrin, molybdate, α-naphthol, and periodate-Schiff reagents. The IR spectrum of the lipid contained all the absorption peaks found in GL1a and PNL1a without additional absorption peaks (Fig. 1c). The molar ratio of phosphorus, glucose, and glycerol was 1 : 2.04 : 1.96.

Chemical degradation of PNGL1 showed the structural relationships of this lipid to GL1a and PNL1a (Scheme 1). Acetolysis caused splitting of the phosphodiester bond to result in an acetylated glycolipid, which was, in turn, converted to GL1a by deacetylation by mild alkaline methanolysis (Fig. 3). Acid methanolysis released PNL1a (Fig. 3). C_{40} tetraether was obtained from PNGL1 by the combination of acetolysis and acid methanolysis. These results indicated that PNGL1 has a phosphoethanolamine moiety at one side of the C_{40} tetraether core and a glucosylglucose moiety at the other side, because neither one of acid methanolysis and acetolysis could cleave both the phosphate moiety and glucose moiety at the same time. Analysis of aqueous methanol-soluble products of BCl_3 treatment gave the same result as in



Glc, glucose; $EtNH_3^+$, ethanolamine; Ac, acetic acid.

Scheme 1. Specific chemical degradation of PNGL1 into PNL1a, GL1a, and C_{40} tetraether.

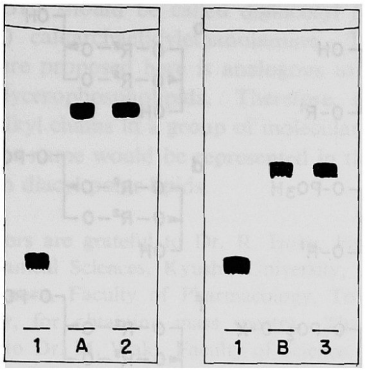


Fig. 3. TLC analysis of the chloroform-soluble products derived from PNGL1 by the combination of acetolysis and mild alkaline methanolysis (A) or by acid methanolysis (B). The TLC plate was developed with solvent B. 1, PNGL1; 2, GL1a; 3, PNL1a.

the case of PNL1a (Table I). HF hydrolysis degraded 79% of the original PNGL1 to yield ethanolamine and NH_3 (40 and 64 mol% of the degraded PNGL1). The structure of the glucosyl-glucose residue was shown to be the same as that of GL1a by partially methylated alditol acetate analysis and CrO_3 oxidation. These results confirmed that PNGL1 had the polar head groups of both GL1a and PNL1a. The positive ion FAB-mass spectrum showed peaks of *m/z* 1,749.4 ($[M+1]^+$) and 1,748.7 ($[M]^+$) which agreed with the molecular weight (1,748.4) expected from the structure shown in Fig. 2c. Finally it was concluded that PNGL1 is C_{40} tetraether having phosphoethanolamine and β-glucopyranosyl-(1-6)-β-glucopyranosyl residues at each side of the core lipid (proposed name: gentiobiosyl caldarchaetidyl-ethanolamine).

DISCUSSION

The structures of two ethanolamine-containing lipids and one glycolipid were elucidated. PNL1a and PNGL1 are the first examples of ethanolamine-containing tetraether lipids in archaebacteria, although ethanolamine is found widely in the lipids of eubacteria and eukaryotes. Because ninhydrin-positive lipids with mobilities identical to those of PNL1a and PNL2b on a TLC plate have been found in *Methanobacteriaceae* and *Methanosarcinaceae* (*11*), it is concluded that ethanolamine-phospholipids and serine-phospholipids are widely distributed in methanogens. On the other hand, ethanolamine- or, more broadly, amino group-containing lipids have rarely been detected and none has been identified in any other branch of archaebacteria. The presence of these amino group-containing lipids may reflect a history of methanogens which have cohabited with eubacteria and eukaryotes having amino group-containing lipids.

PNGL1, PNL1a, and GL1a form structurally a quartet of lipids along with bare C_{40} tetraether, which was present as a neutral lipid in the total lipid extract (*3*). Similar examples of two quartets of tetraether lipids were found in *Sulfolobus solfataricus* (*Caldiella acidophyla*) (*12*) and an incomplete quartet was found in *M. hungatei* (*1*). The "quartet" means here 4 tetraether lipids naturally present in one organism, one of which possesses two different polar head groups (one of them contains a phosphate moiety and the other is a glycoside) and the other two of which each have one polar group of the two. The last of the four contains no polar head group, that is, it is the bare C_{40} tetraether. The fact that the tetraether core is bifunctional makes it possible to form a quartet. From a different point of view, it can be considered that the quartet is a result of a random combination of two different polar lipids with C_{20} diether core lipid. It is noteworthy that there is no example so far in which a tetraether lipid has identical polar head groups on both sides. In addition to the tetraether quartet, 2 diether lipids with the respective polar groups and a diether without any polar groups will form a "heptad" which would be expected to be found in methanogens. C_{20} diether is naturally present in the total lipid extract (*3*) and diglucosyl C_{20} diether has already been found in our laboratory in *M. arboriphilus* A2 strain; it cochromatographed with GL1b of *M. thermoautotrophicum* (data not shown). The structural features of the heptad should provide useful clues for elucidation of the biosynthesis of tetraether polar lipids.

BCl_3 treatment is a classical method for cleaving of ether bonds. Although this reaction destroys glycosidic linkages, a phosphodiester linkage is not affected. As a result, glycerophosphoethanolamine was recovered from PNL1a and PNGL1. This provides convincing evidence for structural

characterization of archaebacterial phospholipids, just as mild alkaline methanolysis was used in the case of diester phospholipids. The recovery of glycerophosphoethanolamine was almost 100%, superior to that in the case of HF hydrolysis in the structure determination of phosphate-containing polar head groups.

Although recently a number of archaebacterial polar lipids with ether bonds have been found, there are no systematic trivial names for them. Because terms such as "a diphytanyl ether analog of phosphatidylserine" (PNL2b) or "a phosphoethanolamine derivative of diglucosyl tetraether" (PNGL1) are too lengthy, authors tend to use random laboratory designations (for example, PNL1a, *etc.*). Of course, such laboratory designations are merely symbols or numbers, and do not have general significance. Since nearly 20 polar lipids from archaebacteria have been structurally characterized to date, we need to have a series of trivial names of archaebacterial lipids. We propose here a nomenclature of archaebacterial ether lipids.

Archaeol—2,3-Di-*O*-alkyl *sn*-glycerol diether (Fig. 4a). Glycerol may be replaced by other polyols, such as tetritol. In the case of glycerol, the fact that alkyl chains are bound at *sn*-2 and *sn*-3 positions is absolutely necessary for an ether lipid to be an archaeol. Alkyl chains are usually isoprenoids with 15, 20, or 25 carbons.

Caldarchaeol—Two molecules of glycerol or other polyols are bridged by 2 molecules of alkanediols (usually poly-isoprenoid diols) through ether linkages which form a tetraether (Fig. 4b). Ether bonds are located at the *sn*-2 and *sn*-3 positions if the polyol is glycerol. A name such as diglycerocaldarchaeol or glycerononito-caldarchaeol is possible. The name is given because caldarchaeol is a predominant core lipid of thermophilic archaebacteria (the prefix "cald" is taken from the Latin word "calidus," meaning warm). Caldarchaeol may also include such a compound as a trialkyl diglycerol tetraether which is made up from two phytanyl chains, one C_{40} isoprenoid and two glycerols.

Archaetidic Acid and Caldarchaetidic Acid—A monophosphate ester of archaeol and caldarchaeol (Fig. 4, c and d). The former is a diether analog of phosphatidic acid. Though the presence of these lipids in the archaebacteria has not been

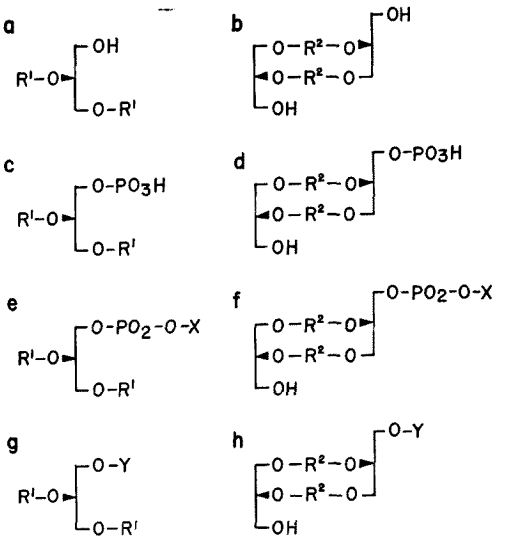


Fig. 4. Proposed nomenclature of di- and tetraether lipids from archaebacteria. R^1 is an alkyl (usually C_{15}, C_{20}, or C_{25} isoprenoid) group; R^2 is an alkanediyl (usually isoprenoid diyl) group, for example, with 40 carbons with or without cyclopentane rings. X represents a polar alcohol such as serine, ethanolamine, inositol, glycerophosphate, or glycerol. Y represents the glycoside chain. a, archaeol; b, caldarchaeol; c, archaetidic acid; d, caldarchaetidic acid; e, archaetidyl X; f, caldarchaetidyl X; g, glycosyl archaeol or glycosyl dialkylglycerol; h, glycosyl caldarchaeol.

reported, these names are important because these are the basic starting compounds of various phosphate-containing lipids from a nomenclature point of view.

Phosphodiester of Archaetidic Acid or Caldarchaetidic Acid—Archaetidic acid or caldarchaetidic acid and an alcohol are linked through a phosphodiester bond (Fig. 4, e and f). The alcohol may be serine, ethanolamine, glycerol, glycerophosphate, inositol, and so on. These lipids are named as derivatives of archaetidic acid or caldarchaetidic acid. For instance, archaetidylserine is a diether analog of phosphatidylserine (PNL2b in *M. arboriphilus* A2 (*2*)). PNL1a is, thus, called caldarchaetidylethanolamine.

Glycosyl Archaeol and Glycosyl Caldarchaeol—A glycoside residue is bound to archaeol or caldarchaeol at a free hydroxyl group through a glycosidic linkage (Fig. 4, g and h).

PNGL1 should be called diglucosyl (or gentiobiosyl) caldarchaetidylethanolamine. The nomenclature proposed here is analogous to that of diacyl glycerophospholipids. Therefore, a variation of alkyl chains in a group of molecular species under one name would be represented in the same way as in diacyl polar lipids.

The authors are grateful to Dr. R. Isobe, Faculty of Pharmaceutical Sciences, Kyushu University, and Dr. A. Tokumura, Faculty of Pharmacology, Tokushima University, for obtaining mass spectra. Thanks are also due to Dr. M. Waki, Faculty of Science, Kyushu University, for his help in measuring optical rotations, and to K. Shimamoto for the amino acid analysis.

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