Structures of Archaebacterial Membrane Lipids

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Structural data on archaebacterial lipids is presented with emphasis on the ether lipids of the methanogens. These ether lipids normally account for 80–95% of the membrane lipids with the remaining 5–20% of neutral squalenes and other isoprenoids. Genus-specific combinations of various lipid core structures found in methanogens include diether–tetraether, diether–hydroxydiether, or diether–macrocyclic diether–tetraether lipid moieties. Some species have only the standard diether core lipid, but none are known with predominantly tetraether lipids as found in certain sulfur-dependent archaebacteria. The relative proportions of these lipid cores are known to vary in relation to growth conditions in *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*. Polar headgroups in glycosidic or phosphodiester linkage to the *sn*-1 or *sn*-1′ carbons of glycerol consist of polyols, carbohydrates, and amino compounds. The available structural data indicate a close similarity among the polar lipids synthesized within the species of the same genus. Detection of lipid molecular ions by mass spectrometry of total polar lipid extracts is a promising technique to provide valuable comparative data. Since these lipid structures are stable within the extreme environments that many archaebacteria inhabit, there may be specific applications for their use in biotechnology.

KEY WORDS: Ether lipid structures; lipid cores; squalenes; isoprenoids; methanogens; archaebacteria; biotechnology.

INTRODUCTION

Advancements in the structural elucidation of archaebacterial lipids has been slow for several reasons. With the exception of aerobic extreme halophiles, difficulties are often experienced to generate the 100–500 g (wet cell weight) of biomass needed to provide sufficient pure lipid for analysis, especially from the obligately anaerobic methanogens, and the aerobic and anaerobic sulfur-dependent extreme thermophiles (Kelly and Deming, 1988). Purification of a lipid may be difficult considering that 15 or more (Sprott *et al.*, 1991) ether lipids may be present in the polar fraction and that the ether lipid of interest may be a minor component. Usually the lipid fraction is only about 5% of the cell dry weight. Also, the chemical methodologies in common use may in some cases be

inadequate or result in the generation of artifacts from uncharacterized, labile components (Ferrante *et al.*, 1988a). Fortunately, the richness of new isolates of archaebacteria as source for new lipid structures of potential use in biotechnology applications provides ample incentive to overcome these difficulties.

Diether lipids were first discovered by Kates and associates (reviewed by Kates, 1978) to comprise the major portion of the total lipids of the extreme halophile *Halobacterium cutirubrum*. Following the discovery of a tetraether structure in *Thermoplasma acidophilum* (Langworthy, 1977), a predominance of ether lipids has proven to be a characteristic of archaebacteria (Makula and Singer, 1978; Tornabene and Langworthy, 1979), distinguishing this grouping of organisms from the eubacteria and eukaryotes. The ether lipid core structure of *H. cutirubrum* was fully elucidated as 2,3-diphytanyl-*sn*-glycerol, of opposite stereochemistry to the glycerolipids of non-archaebacteria (Kates, 1978, 1988). Several variations of this

"standard diether" are found among the divergent members of the archaebacteria, but all retain the fully saturated, branched phytanyl chains of 5-carbon repeating units and the sn-2,3-diphytanyl stereochemistry.

Similar to the finding that factor F420 is not exclusively found in methanogens (Eker et al., 1980), there are exceptions also in archaebacterial lipid chemistry. For example, rarely eubacterial obligate anaerobes contain plasmalogen glycerophospholipids and glycerol diethers (Langworthy et al., 1982). Even the archaebacterial R configuration is found in a minor monoether geranylgeranylglycerol lipid from the brown algae Dilophus fasciola (Amico et al., 1977).

The nomenclature of ether lipids used here[2] is simple and one immediately descriptive of structure. Diether (D_s) and tetraether (T_s) are used for the standard diphytanylglyceroldiether and dibiphytanyl-diglyceroltetraether, sometimes referred to as archaeol and caldarchaeol (Nishihara et al., 1987). Structural variations are shown as prefixes to the standard structures, such as $C_{20,25}$-diether to denote C_{20} and C_{25} alkyl chains at the sn-3 and sn-2 carbons of glycerol, respectively.

Archaebacterial lipids have provided a subject worthy of numerous reviews (DeRosa and Gambacorta, 1988; De Rosa et al., 1986a; Kamekura and Kates, 1988; Kates, 1972, 1978, 1988, 1990. Kates and Kushwaha, 1978; Kates et al., 1982; Langworthy, 1978, 1982, 1985; Langworthy and Pond, 1986; Ross et al., 1985). Here the focus is on recent advances in the structural elucidation of methanogen ether lipids with comparison to the ether lipids discovered in other archaebacteria. The formation of ether lipid vesicles is briefly described, as well.

LIPID CORE STRUCTURES

A prudent choice of hydrolytic conditions leads to the release of the polar headgroup from the ether lipid core. The usual methods (Kates, 1986) are 2.5% methanolic-HCl for 2 h at 70°C (glycolipids) and either ice-cold 48% hydrofluoric acid overnight or mild alkaline methanolysis (phospholipids). There is

as yet no universally acceptable hydrolysis method. Core lipids are not always released with an expected free hydroxyl group remaining on the sn-1 carbon of glycerol. For example, acid methanolysis of an M. voltae diether in phosphodiester linkage to N-acetylglucosamine resulted in release of the phosphorylated form of the diether (Ferrante et al., 1986). Similarly, a phosphatidylserine diether which lacks a free hydroxyl group adjacent to the phosphodiester bond was not readily degraded by acid methanolysis, requiring acetolysis to yield the diether lipid core (Morii et al., 1986). Attempts to use phospholipase C or D has met with success to release a phosphoinositol headgroup (Morii et al., 1988), but not phosphoserine (Morii et al., 1986). Other more serious problems are encountered if the lipid core is degraded during chemical hydrolysis. Generally, these difficulties are not appreciated when archaebacteria are classified according to the types of lipid cores present.

Hydrolysis of total (or polar) lipids releases lipid cores for analysis by TLC or other methods such as high-performance liquid chromatography (Mancuso et al., 1986) or capillary supercritical fluid chromatography (DeLuca et al., 1986). The standard diether structure found in H. cutirubrum (Kates, 1978) is ubiquitous among methanogens. All methanogens analyzed to date contain standard diether and sometimes a modified diether with from 0 to about 50% of the standard tetraether (Fig. 1). Diether modifications are the macrocyclic diether found only in M. jannaschii (Comita et al., 1984), and the hydroxydiethers found in Methanosaeta ("Methanothrix") concilii (Ferrante et al., 1988a) and Methanosarcina barkeri (Sprott et al., 1990; Nishihara and Koga, 1991). It is unclear whether glycerol monoethers are present as trace components or as artifacts of hydrolysis procedures (Sprott et al., 1990). Other archaebacterial diether modifications are not yet discovered in methanogens including the $C_{20,25}$-diether (DeRosa et al., 1982) and $C_{25,25}$-diether (DeRosa et al., 1983) which were screened for and found among certain extreme halophiles (DeRosa and Gambacorta, 1988). The core lipids of Sulfolobus sulfataricus and Thermoplasma acidophilum are 90 to 95% tetraethers and 5-10% standard diether (Langworthy, 1982). In addition to the methanogen T_s structure (Fig. 1), the tetraether C_{40} chains of Thermoplasma and certain sulfur-dependent thermophiles may be modified to contain from 1 to 4 cyclopentane rings and/or to be dibiphytanylglycerolnonitoltetraethers. Also, glycerol-trialkyl-glycerol tetraether or monophytanylglycerol

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[2]Abbreviations: D_s, standard diether, archaeol or 2,3-di-O-phytanyl-sn-glycerol; T_s, standard tetraether, caldarchaeol or 2,2',3,3'-tetra-O-dibiphytanyl-sn-diglycerol; D_{OH}, 3- or 3'-hydroxy-diether; D_M, macrocyclic diether or 2,3-di-O-cyclic-biphytanyl-sn-glycerol; P, phospho moiety; NAc, N-acetyl; E, ethanolamine; I, inositol.
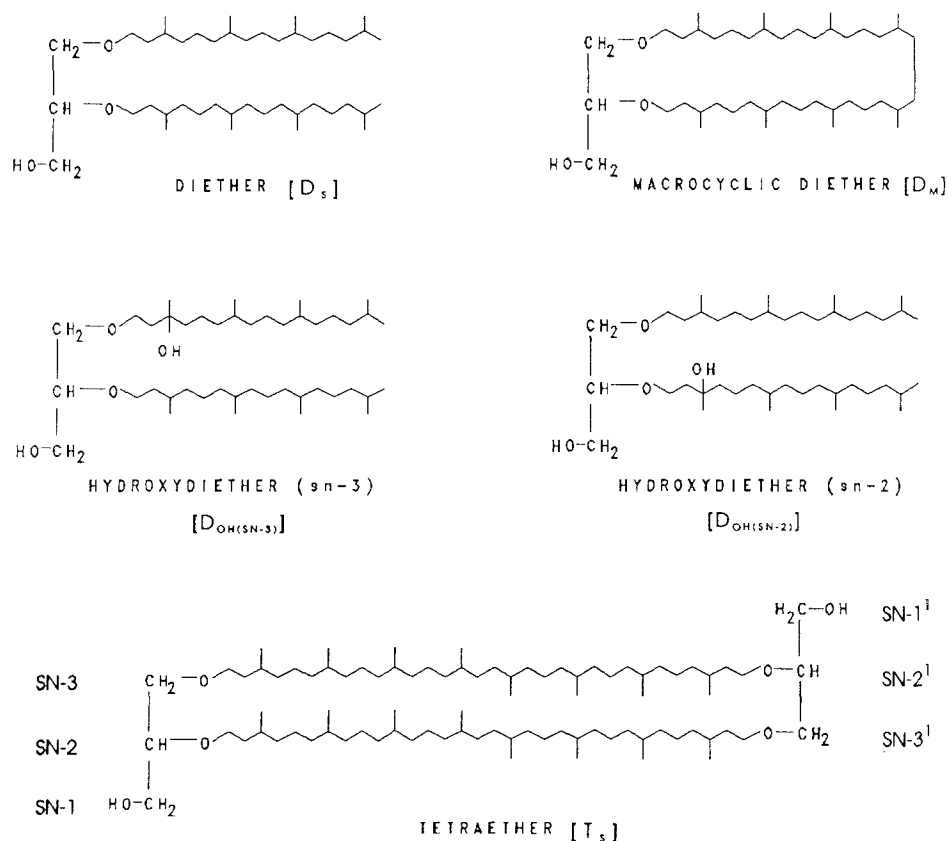
Fig. 1. Lipid core structures found in methanogens.

may be present as minor lipids (De Rosa and Gambacorta, 1988).

Acid-labile 3'-hydroxydiether lipids comprise about 30% and standard diether the remainder of the lipid cores of *Methanosaeta concilii* (Ferrante *et al.*, 1988a). A screening of 15 methanogens established that acid-labile hydroxydiether lipids were present in even higher proportion to the standard diether in *Methanosarcina barkeri* MS and *M. mazei*, where hydroxylation occurred again at C-3 but on the opposite sn-2 alkyl chain (Sprott *et al.*, 1990). The ^{13}C NMR signals of both hydroxydiethers and standard diether have been assigned, characteristic ^1H NMR signals interpreted, and $-FAB$ MS plus optical rotations (M_D + 35.1 to +39.8°) measured to confirm the structures (Ferrante *et al.*, 1988a; Sprott *et al.*, 1990). Hydroxydiethers were similarly abundant and of the same structure (3-hydroxyl on sn-2 chain) in *M. barkeri* Fusaro, and *M. acetivorans* grown on methanol, acetate, or CO_2/H_2 (Sprott, G. D., Patel, G. B., Ekiel, I., and Choquet, C., unpublished data), suggesting this to be of taxonomic relevance. However, other methanogens including *Methanococcus voltae* and several *Methanobacterium* spp. also have hydroxydiethers, albeit in much lesser amounts. These latter structures have yet to be characterized fully (Sprott *et al.*, 1990).

Strong acidic hydrolysis of hydroxydiethers results in its conversion to monophytanylglycerol (loss of the chain which is hydroxylated), and to three major components corresponding to 3-(or 3') methoxydiether and cis/trans isomers unsaturated between carbons 3 and 4 (Ferrante *et al.*, 1988a; Sprott *et al.*, 1990; Ekiel and Sprott, 1992). The latter cis/trans isomers appear as a double spot resembling the mobilities on TLC of $C_{20,20}$ and $C_{20,25}$-diethers, and would account for the incorrect assumption based on TLC evidence alone that $C_{20,25}$ lipid cores are synthesized by *Methanosarcina* spp. (Grant *et al.*, 1985; De Rosa and Gambacorta, 1988). The results of DeRosa *et al.* (1986b) reporting a wide range of physiological lipid cores in *M. barkeri* MS could not be repeated either (Sprott *et al.*, 1990; Nishihara and Koga, 1991), suggesting the need, in hindsight, for caution when

characterizing strains potentially containing hydroxy-diethers.

Any attempts to analyze the ether lipids from methane-generating environmental samples or anaerobic reactors, where *Methanosaeta* spp. and *Methanosarcina* spp. are normally present in high numbers, requires that mild hydrolysis be performed. This has not been recognized in past studies (Hedrick *et al.*, 1991), but in future is likely to provide the basis for valuable additional information on the aceticlastic activity of methanogenic bioreactors or sediment samples.

The hydroxydiether is itself not an artifact, since NMR analysis of total polar lipid extracts clearly identifies this as a native structure (Sprott *et al.*, 1990). Headgroup removal from the polar lipids of *M. concilii* or *Methanosarcina* spp. by mild methanolic-HCl or cold hydrofluoric acid results in minimal degradation of these sensitive lipids (Ferrante *et al.*, 1988a).

GROWTH CONDITIONS AFFECT LIPID CORE DISTRIBUTIONS

Some archaebacteria are capable of growth over a wide range of growth conditions, yet their polar lipids are fully saturated, consistently branched phytanyl chains, with limited to no variability in alkyl chain length. How then do these organisms maintain the liquid crystalline state of their membrane lipids necessary for growth? *Sulfolobus sulfataricus* synthesized tetraether lipids with increasing numbers of pentacyclic rings in response to increases in growth temperature in the range 75–89°C (DeRosa *et al.*, 1980). The physical consequences of the shortening of membrane-spanning tetraether lipids by introduction of pentacyclic rings has been discussed (DeRosa *et al.*, 1986a; Kates, 1988). An alkalophilic extreme halophile *Natronococcus occultus* grown in increasing salt concentrations resulted in a decline of a phosphatidylglycerol $C_{20,20}$-diether (PG, see Table II) and in a corresponding elevation in a phosphatidylglycerophosphate $C_{20,25}$-diether (Nicolaus *et al.*, 1989).

In methanogens it now appears that more than one lipid core is common, thus allowing for the possibility of alterations in their proportions in response to growth conditions. In the deep-sea thermophile *Methanococcus jannaschii* dramatic shifts from a predominantly standard diether membrane to tetraether and macrocyclic diethers occurred as the growth temperature increased from 47 to 75°C (Sprott *et al.*,

1991). Cyclization of the alkyl chains is expected to decrease their freedom of motion and contribute to an acceptable membrane at high temperatures. C_{40}-tetraethers should stabilize the membranes by forming covalently bonded bilayers (monolayers) that span the membrane. Evidence for this type of monolayer organization has been presented for thermoacidophiles (DeRosa *et al.*, 1986a). Variation of the tetraether content of *M. jannaschii* from about 10 to 45% of the total lipid cores results in a dramatic decline in hydrophobic membrane cleavage planes, supporting a monolayer organization of the tetraethers (Beveridge, T. J., and Sprott, G. D., unpublished data). A second example of shifts in diether to tetraether ratios occurs in response to the growth phase of *Methanobacterium thermoautotrophicum* (Kramer and Sauer, 1991).

Extreme thermophiles in the *Methanothermaceae* generally contain tetraether lipids (and standard diether) as is the case for *Methanothermus* spp. (Lauerer *et al.*, 1986).

However, *Methanopyrus kandlerii* grows optimally at 98°C, yet the membrane lipids are exclusively diethers (Kurr *et al.*, 1991), showing that generalizations to explain membrane integrity are not yet possible. Similar exceptions in absence of tetraether are found in *Pyrococcus woesei*, AN1 (Lanzotti *et al.*, 1989c) and *Thermococcus celer* (DeRosa *et al.*, 1987). Research is needed to define the importance of lipid–protein interactions to understand membrane stability and function during growth in harsh environments.

POLAR LIPIDS OF METHANOGENS

The first polar lipid structures of a methanogen were presented for *Methanospirillum hungatei* (Kushwaha *et al.*, 1981), and later expanded to include the lipids with unusual N,N-dimethyl and N,N,N-trimethyl moieties (Ferrante *et al.*, 1987; Ferrante, G., Ekiel, I., and Sprott, G. D., unpublished data). The lipids are derivatives of the standard tetraether and diether cores consisting of four major components (PGL-1, PGL-2, PPDAD, and PPTAD) and numerous minor lipids of < 5 wt.% each (Fig. 2). Glycosidic headgroup moieties are repetitious in the sense that the first sugar residue attached glycosidically *sn*-1 to glycerol is β-Gal$_f$ and the second either β-Gal$_f$ or α-Glc$_p$. The *sn*-1′ carbon of glycerol of tetraethers is either bare (–OH) or phosphorylated to a polyol, with the minor lipid TGT-1 as exception. The N,N-dimethyl and N,N,N-trimethylaminopentanetetrol
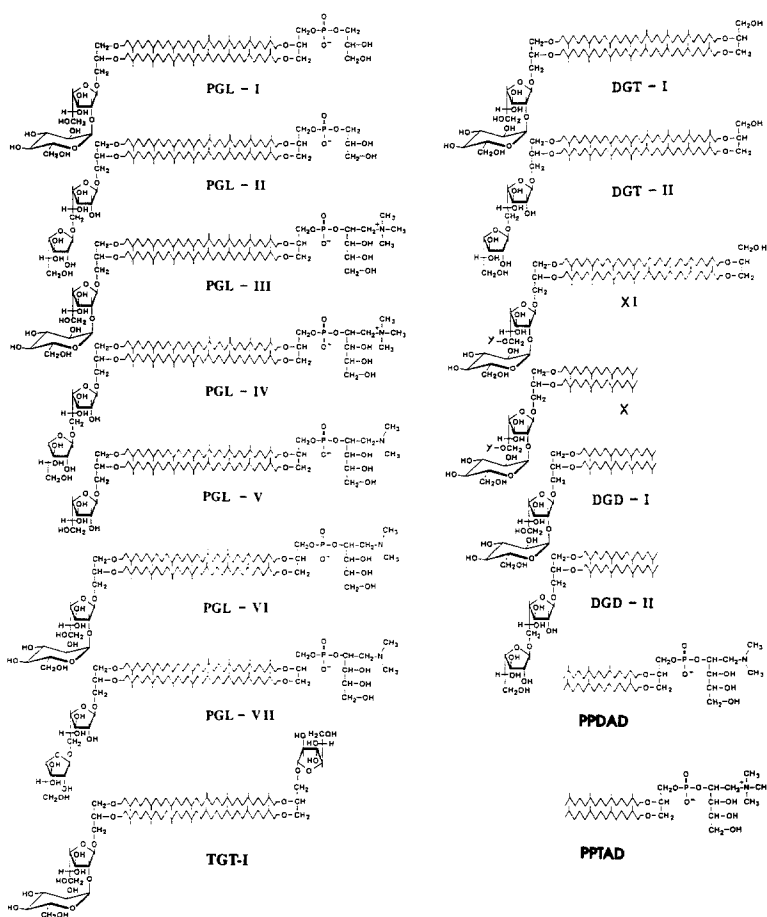
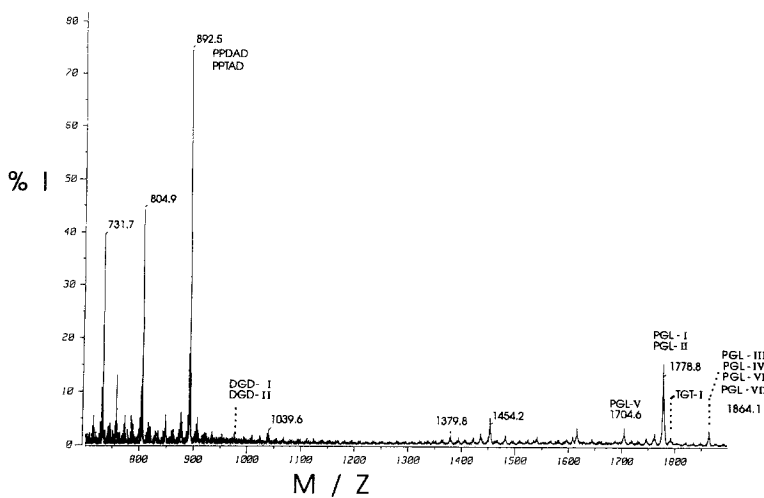**Fig. 2.** Polar lipids of *Methanospirillum hungatei* GP1.

Fig. 3. Negative-FAB MS spectrum of the total polar lipids from *Methanospirillum hungatei* GP1. The spectrum was acquired with a JEOL (JMS-AX505H) instrument with triethanolamine matrix.

Table I. Polar Lipids of Methanogens

Methanogen	Structure	Percent of polar lipids	Reference
Methanococcus voltae	β-Glcp-(1-6)-β-Glcp-(1-1)-D$_s$	63	Ferrante *et al.*, 1986
	β-Glcp-D$_s$	3	Ferrante *et al.*, 1986
	β-GlcpNAc-P-D$_s$	7	Ferrante *et al.*, 1986
	P-D$_s$	< 1	Ferrante *et al.*, 1986
Methanobacterium thermoautotrophicum	β-Glcp-(1-6)-β-Glcp-(1-1)-T$_s$	16	Nishihara *et al.*, 1987
	β-Glcp-(1-6)-β-Glcp-(1-1)-T$_s$-P-E	7	Nishihara *et al.*, 1987
	β-Glcp-(1-6)-β-Glcp-(1-1)-T$_s$-P-I	—	Nishihara *et al.*, 1989
	β-Glcp-(1-6)-β-Glcp-(1-1)-T$_s$-P-Ser	—	Nishihara *et al.*, 1989
	T$_s$-P-E	9	Nishihara *et al.*, 1987
	T$_s$-P-I	—	Nishihara *et al.*, 1989
	T$_s$-P-Ser	—	Nishihara *et al.*, 1989
	β-Glcp-(1-6)-β-Glcp-(1-1)-D$_s$	—	Nishihara *et al.*, 1989
	D$_s$-P-E	3	Nishihara *et al.*, 1989; Kramer *et al.*, 1987
	D$_s$-P-I	—	Nishihara *et al.*, 1989
	D$_s$-P-Ser	—	Nishihara *et al.*, 1989
	P-D$_s$	—	Nishihara and Koga, 1990
	P-T$_s$	—	Nishihara and Koga, 1990
Methanosaeta concilii	α-Manp-(1-3)-β-Galp-(1-1)-D$_s$	39	Ferrante *et al.*, 1988b
	β-Galp-(1-6)-β-Galp-(1-1)-D$_{OH}$	20	Ferrante *et al.*, 1988b
	β-Galp-D$_s$	< 2.1	Ferrante *et al.*, 1989
	β-Galp-D$_{OH}$	< 2.1	Ferrante *et al.*, 1989
	β-Galp-(1-6)-[β-Glcp-(1-3)]-β-Galp-D$_{OH}$	0.3	Ferrante *et al.*, 1989
	D$_s$-P-E	4	Ferrante *et al.*, 1989
	D$_{OH}$-P-E	9	Ferrante *et al.*, 1989
	D$_s$-P-I	24	Ferrante *et al.*, 1988b
Methanobrevibacter arboriphilicus	β-Glcp-(1-6)-β-Glcp-T$_s$	10	Morii *et al.*, 1988
	β-Glcp-(1-6)-β-Glcp-T$_s$-P-I	30	Morii *et al.*, 1988
	T$_s$-P-I	11	Morii *et al.*, 1988
	β-Glcp-(1-6)-β-Glcp-D$_s$	13	Morii *et al.*, 1988
	D$_s$-P-I	3	Morii *et al.*, 1988
	D$_s$-P-Ser	35	Morii *et al.*, 1986
Methanosarcina barkeri	D$_{OH}$-P-I	24	Nishihara and Koga, 1991
	D$_s$-P-I	1	Nishihara and Koga, 1991
	D$_{OH}$-P-Ser	17	Nishihara and Koga, 1991
	D$_s$-P-Ser	2	Nishihara and Koga, 1991
	D$_s$-P-I-6lan	24	Nishihara *et al.*, 1992

headgroups are presently restricted to *M. hungatei* spp.

A −FAB MS analysis of the total lipids of *M. hungatei* (Fig. 3) illustrates the difficulties that can be encountered in attempting strain comparisons by this method. Several lipids have the same molecular weight and consequently produce a single molecular ion signal. Phosphatidyl diether and phosphatidyl tetraether have been found as minor lipids in certain other methanogens (Table 1), but during −FAB MS analysis of total polar lipids these would appear also as normal fragmentation products. Also, PPTAD is detected as the demethylated form with an m/z identical to PPDAD (Ferrante *et al.*, 1987) requiring a +FAB analysis to distinguish these lipids. Finally, the sensitivity to tetraether lipids is less than to diethers (PGL-1 is the most abundant lipid), and sensitivity is low for lipids lacking a phosphate moiety. The method was useful in revealing a 804.9 signal which is suspected to correspond to the phosphatidylglycerol-diether (PG) found in extreme halophiles; however, further support is needed since a −FAB MS analysis
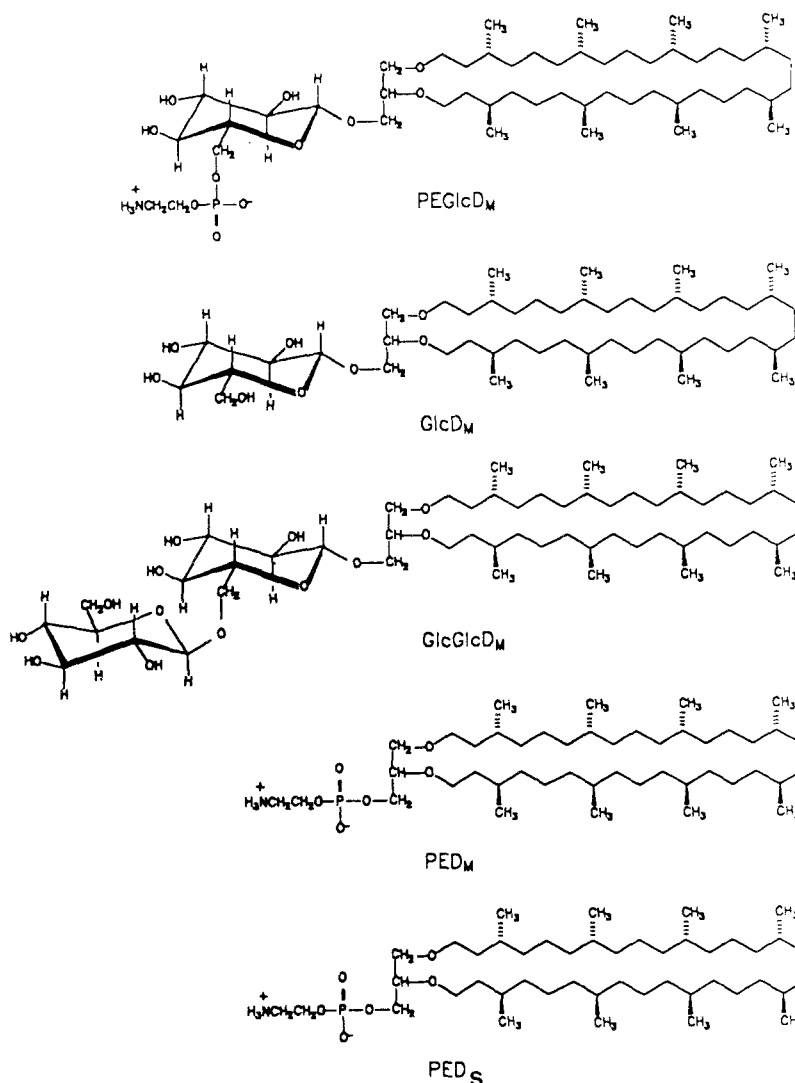
Fig. 4. Structures of several major lipids of *Methanoccoccus jannaschii*. PE, phosphoethanolamine. Data from Ferrante *et al.* (1990).

of PG from *H. cutirubrum* produces an *m/z* of 805.6 of 1 H higher mass (Ferrante *et al.*, 1987; Fredrickson *et al.*, 1989).

A summation of the polar lipid structural data for *M. jannaschii* and other methanogens is presented in Fig. 4 and Table I. Some genera contain the ether lipid analogs of phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine. Most synthesize glycolipids of 1 to 3 sugars often exclusively of *D*-glucose, although glucose and galactofuranose lipids are common in *M. hungatei*, and glucose, galactose, and mannose in *M. concilii*. The β-Glc$_p$-(1-6)-β-

Glc$_p$-headgroup repeats often and is common to *M. voltae*, *M. jannaschii*, *M. arboriphilicus*, and *M. thermoautotrophicum*. A major aminophosphoglycolipid novel to *M. jannaschii* was unusual in exhibiting a phosphodiester linkage of ethanolamine at glucose C-6 (Fig. 4). Numerous other diether and tetraether lipids found in this methanogen have yet to be analyzed (Sprott *et al.*, 1991).

The polar lipids of *Methanosarcina barkeri* contrast to the others described since they are primarily phospholipids, with only a single glycolipid accounting for 6 mol% of the total (Nishihara and Koga,
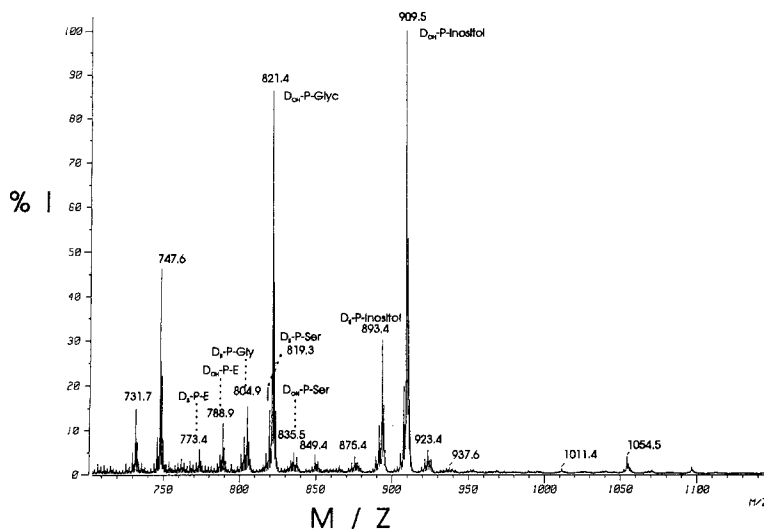
Fig. 5. Negative FAB MS spectrum of the total polar lipids of *Methanosarcina mazei*
S6. Conditions as in legend to Fig. 3. Gly, tentatively glycerol; Glyc, glycerol.

1991). These lipids are ideal for −FAB analysis of total polar lipid extracts, shown for *M. mazei* grown on methanol (Fig. 5). Immediately the phosphatidyldiether (731.7) and phosphatidylhydroxydiether (plus an oxygen atom) fragments are seen (Ferrante *et al.*, 1988a), which provide useful structural data for uncharacterized, purified lipids. In addition to the structures reported by Nishihara and Koga (1991) in Table I, several other structures were assigned and confirmed by ^{13}C NMR and optical rotations of the purified lipids. Assignment of the 804.9 molecular ion as phosphatidylglycerol diether is tentative at present, but the phosphatidylglycerol hydroxydiether form was positively identified by ^{13}C NMR and MS. Also, comparison by −FAB MS of polar lipid extracts from several *Methanosarcina* spp. revealed that the same lipids were present regardless of species or of the growth substrates (Sprott, G. D., Ekiel, I., Dicaire, C., and Patel, G. B., unpublished data).

POLAR LIPIDS OF EXTREME HALOPHILES

The polar ether lipids of *Halobacterium* spp. are standard diethers consisting of one major phospholipid (PGP), two minor phospholipids (PG and sulfated-PG), and one major (sulfated-Gal-Man-Glc-D_s) plus several minor glycolipids (Table II). Ethanolamine, serine, and inositol headgroups are absent. The strain-dependent headgroup variations found in extreme halophiles have been reviewed in detail (Kates, 1988, 1990).

Alkalophilic extreme halophiles such as *Natronobacterium* spp. and *Natronococci* spp. differ from *Halobacteria* spp. by the absence of sulfated-PG and glycolipids (Morth and Tindall, 1985; Kates, 1990). Both $C_{20,20}$- and $C_{20,25}$-diether derivatives of PG and PGP are major lipids. *Natronococcus occultus* produces these lipids and a novel variation of the $C_{20,20}$ and $C_{20,25}$ PGP where the terminal phosphate is bonded as $1',2'$-cyclic phosphate (Lanzotti *et al.*, 1989a). Also, novel to the haloalkalophiles is the association between trimethylglycine (glycine betaine) and PGP (DeRosa *et al.*, 1988).

In contrast to the extreme halophiles, sulfation has not been found in methanogen glyco- or phospholipids, nor have the same glycolipid structures been observed. Phosphatidylglyceroldiether (PG) and phosphatidyldiether (P-D_s) appear to be the only polar lipids which overlap between methanogens and extreme halophiles. PG is ubiquitous among extreme halophiles but is reported above only in *Methanosarcina* spp. (as the D_{OH} form) and tentatively in *Methanospirillum hungatei*. P-D_s is found in an extreme halophile strain 54R isolated from a salt mine (Lanzotti *et al.*, 1989b) and may prove to be a minor lipid common to many archaebacteria, based on the repeating phosphatidyl ether moiety found in many of the polar lipids (Table I).

Table II. Polar Lipids of Extreme Halophiles[a]

Lipid Class	Structure
Phospholipids	P-Glyc-P-D$_s$ (PGP)
	Glyc-P-D$_s$ (PG)
	HSO$_3$-Glyc-P-D$_s$ (PGS)
Glycolipids	3-HSO$_3$-β-Galp-(1-6)-α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	β-Galp-(1-6)-α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	6-HSO$_3$-α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	β-Glcp-(1-6)-α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	3-HSO$_3$-β-Galp-(1-6)-α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	|
[(3-1)-β-Gal$_f$]
	β-Galp-(1-6)-α-Manp-(1-2)-α-Glcp-(1-1)-D$_s$
	|
[(3-1)-β-Gal$_f$] |

[a]Note the repetitive nature of sugar residues and linkages.

POLAR LIPIDS OF *Thermoplasma* AND THERMOPHILIC SULFUR-DEPENDENT ARCHAEBACTERIA

Because *Thermoplasma* is no closer phylogenetically to *Sulfolobus* and its relatives than it is to the methanogens (Stetter and Zillig, 1985), a comparison of ether lipid structures is interesting. *Thermoplasma acidophilum* contains a tetraether lipid accounting for 80% of the polar lipids which is not particularly distinguishing; it has a single sugar at one end and phosphoglycerol at the other (Table III). In addition, 3% of the polar lipids is a lipopolysaccharide rich in mannose. It is unknown whether this latter novel structure is a distinguishing characteristic or reflects a lack of effort to screen other archaebacteria for this class of lipid.

Sulfolobus sulfataricus is one of the thermophilic archaebacteria in the *Sulfolobales* typically rich in nonitol tetraethers (DeRosa and Gambacorta, 1988). Residues are attached to the nonitol moiety of the tetraether through C-3', and both tetraether and nonitol tetraether polar lipids are further complicated by growth condition-dependent cyclizations within the C$_{40}$ chains (reviewed in DeRosa *et al.*, 1986a; Gulik *et al.*, 1988). One lipid was sulfated reminiscent of the extreme halophiles, but this seems to be more rare among this group.

The glycolipids of *Thermoproteus tenax* are composed of glucose residues linked to tetraethers which have from 0 to 5 cyclopentane rings (not shown), similar to *Sulfolobus*.

Two *Pyrococcus* spp. each had two major lipids, one representing a novel archaebacterial lipid where phosphorylation occurred at the C-3 of glucose. A phosphatidylinositoldiether was a major lipid in both *Pyrococcus* spp. and *Thermococcus celer* where it represented 80% of the total lipids (DeRosa *et al*, 1987).

Scrutiny of this limited structural data suggests that only the species with nonitol tetraether and/or cyclopentanetetraether core lipids are very different from methanogens, although additional variations within headgroups may aid in definition at the genus level.

NEUTRAL LIPIDS

Neutral lipids usually constitute about 10 wt.% of the total extractable lipids. *Thermoplasma, Sulfolobus* spp., and a number of methanogens contain predominantly acyclic isoprenoid and hydroisoprenoid hydrocarbons with C$_{30}$ most abundant, and a series of straight-chain alkanes ranging from C$_{14}$ to C$_{36}$ (Holzer *et al.*, 1979, 1988; Tornabene *et al.*, 1979). In addition, ether lipid cores (T$_s$ and/or D$_s$) were identified among the neutral lipids of *Methanospirillum hungatei* (Kushwaha *et al.*, 1981) and *Methanobacterium thermoautotrophicum* (Nishihara *et al.*, 1989).

Extreme halophiles also contain squalenes (C$_{30}$ isoprenoids) as the major neutral lipids, and other components including vitamin MK-8, retinal, C$_{40}$ carotenoids, C$_{50}$ bacterioruberins, D$_s$, and other minor hydrocarbons (Kates *et al.*, 1982). A trace of fatty acids and a fatty acid synthetase sensitive to high salt concentrations are present in *Halobacterium cutirubrum* (Pugh *et al.*, 1971).

Traces of fatty acids have been detected in several methanogens (Makula and Singer, 1978). These are especially striking in *Methanosphaera stadtmanae* where a broad range of fatty acids make up a large portion (43%) of the neutral fraction (Jones and Holzer, 1991). This methanogen contains an unusual 11-hydroxy-nonadecanoic fatty acid, as well as the common C$_{30}$ isoprenoids (only 7% of the neutral lipids) and traces of tricyclic terpenes.

BIOTECHNOLOGY AND SUMMARY

Archaebacterial lipids with natural resistance to oxidation and esterase may find an advantage over

Table III. Polar Lipids of *Thermoplasma* and Sulfur-Dependent Thermophiles

Bacterium	Structure	Reference
Thermoplasma acidophilum	Hexose-T$_s$-P-Glyc [α-Manp-(1-2)-α-Manp-(1-4)-αManp-(1-3)]$_8$-α-Glcp-(1-1)-T$_s$	Langworthy, 1985; Smith, 1980
Sulfolobus sulfataricus and *Desulfurolobus* *ambivalens*	β-Glcp-β-Galp-T$_s$-P-I β-Glcp-β-Galp-T$_s$ β-Galp-β-Galp-T$_s$ β-Glcp-nonitolT$_s$-P-I β-Glcp-nonitolT$_s$ HSO$_3$-β-Glcp-nonitolT$_s$ T$_s$-P-I	DeRosa *et al.*, 1986a; Gulik *et al.*, 1988 and Trincone *et al.*, 1989
Thermoproteus tenax	Glc-Glc-Glc-T$_s$ Glc-Glc-T$_s$ Glc-T$_s$ Glc-T$_s$P-I	Thurl and Schafer, 1988
Desulfurococcus mobilis	α-Glcp-(1-4)-β-Galp-T$_s$-P-I α-Glcp-(1-4)-β-Galp-T$_s$ β-Galp-T$_s$-P-I	Lanzotti *et al.*, 1987
Pyrococcus spp.	D$_s$-P-I 3-P-α-Glcp-(1-1)-D$_s$	Lanzotti *et al.*, 1989c
Thermococcus celer	D$_s$-P-I	DeRosa *et al.*, 1987
Pyrobaculum islandicum and *Pyrobaculum organotrophicum*	β-Glcp-T$_s$ β-Glcp-(1-2)-β-Glcp-T$_s$ β-Glcp-(1-2)-β-Glcp-(1-2)-β-Glcp-T$_s$ β-Glcp-T$_s$-P-I β-Glcp-(1-2)-β-Glcp-(1-2)-β-Glcp-T$_s$-P-I	Trincone *et al.*, 1992

ester lipids for the preparation of liposomes with enhanced stability and storage characteristics. Multi-lamellar liposomes have been prepared from the ether lipids of *Halobacterium cutirubrum* (Quinn *et al.*, 1986), *Sulfolobus sulfataricus* (Gliozzi *et al.*, 1986), and others. Detergent dialysis (and pressure extrusion) results in the preparation of unilamellar liposomes from the natural mix of total polar lipids found in *Methanococcus jannaschii*, *Methanococcus voltae*, *Methanosarcina mazei*, and *Methanosaeta concilii* (Choquet, C., Sprott, G. D., and Patel, G. B., unpublished data). The liposomes varied in size from about 45 to 130 nm and were suitable for entrapment studies. Large 600-nm liposomes constructed from the major tetraether lipid of *Thermoplasma acidophilum* are relatively resistant to surface-active agents (Ring *et al.*, 1986). Importantly, not all archaebacterial lipids form liposomes. Nonitol tetraether lipids require mixing with at least 25% monopolar lipids to obtain closed vesicles (Lelkes *et al.*, 1983). Considerable interest in these novel lipids is anticipated for this, and other, applications including the construction of novel membranes for separation processes (Bauer *et al.*, 1983).

Unilamellar liposomes have been prepared from select tetraether lipids of *Sulfolobus acidocaldarius* by sonication of a lipid subfraction of intermediate polarity (Elferink *et al.*, 1992). Cytochrome *c*-oxidase was functionally reconstituted by addition to the liposomes of cytochrome *c*-oxidase in 1.5% sodium cholate followed by dialysis, freeze-thawing from liquid nitrogen, and sonication. Further, fusion of the proteoliposomes with membrane vesicles of *Lactococcus lactis* resulted in coupling between a proton-motive force generated by cytochrome *c*-oxidase activity and the active transport of leucine. This represents the first demonstration of preservation of function following the incorporation of an integral eubacterial membrane protein into a tetraether liposome.

The polar lipid structures within the archaebacteria are characterized best among the extreme halophiles, primarily because there appears to be less variability within this phylum. Extreme halophiles may be subdivided on this basis as neutrophile and alkalophile subgroups. Scrutiny of the limited data available for methanogens and sulfur-dependent thermophiles indicates that few predictions of polar lipid

structure can be made for new isolates. Crude comparisons among methanogens based on TLC data (Grant *et al.*, 1985) and MS analysis of total polar lipids of *Methanosarcina* spp. (Fig. 5) suggest that species within a genus may exhibit very similar lipid profiles. Improved MS and NMR instrumentation combined with the availability of numerous new isolates and improved methods to generate sufficient biomass are predicted to lead to the discovery of many more ether lipid structures in the near future.

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