MINI-REVIEW

Structural and physicochemical properties of polar lipids from thermophilic archaea

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Abstract The essential general features required for lipid membranes of extremophilic archaea to fulfill biological functions are that they are in the liquid crystalline phase and have extremely low permeability of solutes that is much less temperature sensitive due to a lack of lipid-phase transition and highly branched isoprenoid chains. Many accumulated data indicate that the organism's response to extremely low pH is the opposite of that to high temperature. The high temperature adaptation does not require the tetraether lipids, while the adaptation of thermophiles to acidic environment requires the tetraether polar lipids. The presence of cyclopentane rings and the role of polar heads are not so straightforward regarding the correlations between fluidity and permeability of the lipid membrane. Due to the unique lipid structures and properties of archaeal lipids, they are a valuable resource in the development of novel biotechnological processes. This microreview focuses primarily on structural and physicochemical properties of polar lipids of (hyper) thermophilic archaea.

Keywords Thermophiles · Archaeal polar lipids · Archaeosomes · Physicochemical properties · Biotechnological applications

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Classification of archaea

Archaea are regarded as one of the three main domains of life, its concept being proposed on the basis of phylogenetic analysis of ribosomal RNA (rRNA) sequences (Woese et al. 1990). This classification has been confirmed by extensive phylogenetic studies on a variety of macromolecules found in the patrimony of these microbes. Recently, the concept that Archaea and Bacteria were differentiated based on their membrane lipids has been advocated (Koga et al. 1998; Peretó et al. 2004; Wachtershauser 2003; William and Michael 2003). Archaeal domain, based on the evolutionary relationships, is divided into two phyla, the Euryarchaeota (from the Greek "euryos" meaning diversity) and the Crenarchaeota (from the Greek "crenos" meaning spring or origin; Fox et al. 1980; Woese and Fox 1977; Woese et al. 1990; Woese 1987). With the rise of the molecular ecology and the discovery of many novel lineages of mesophilic and psychrophilic archaea, a new, third archaeal phylum Thaumarchaeota (from the Greek 'thaumas', meaning wonder) was proposed (Brochier-Armanet et al. 2008).

Cultivated archaea are divided, based on their physiological and metabolic characteristics, into six groups. The first group includes extremely halophilic archaea that thrive in environments with salt concentrations up to 200 g/l. In the second group are those extremely thermophilic archaea that grow optimally at 80ºC or more, with a current record of growth at 121ºC (Kashefi and Lovley 2003). The third group is comprised of acidophilic archaea that thrive in acidic environment often also at elevated temperature. To maintain a near neutral pH, these organisms must withstand proton gradients up to five orders of magnitude (Macalady et al. 2004). The fourth group includes ammonia-oxidizing archaea or nitrifiers that are abundant and important in the open ocean (Wuchter et al. 2006) and in soils (Leininger et

al. 2006). They mostly inhabit surfical sediments that are likely to have low concentrations of O_2, and the open ocean, in which ammonia concentrations are typically an order or more lower than in more replete environments. The fifth group includes methanogens, which are a group of strictly anaerobic archaea characterized by the unique ability to produce methane. These organisms thrive across a broad range of temperature, salinity, and pH. The sixth group is composed of anaerobic methane oxidizers that are closely related to the methanogens and are thought to form syntropic associations with sulfate- or nitrate- reducing bacteria (Raghoebarsing et al. 2006).

Special biochemical adaptations of archaea

Archaea and Bacteria differ in many genetic and biochemical properties. One important distinction between Archaea and Bacteria is the chemical structure of lipids composing the cytoplasmic membrane, which will be discussed later in the chapter. Valentine (2007) hypothesized in his article that the adaptation to chronic energy stress is the crucial factor that distinguishes the Archaea from the Bacteria and that the primary biochemical basis for this adaptation are low-permeability membranes and specific catabolic pathways. Catabolic specificity of Archaea (aerobiosis, fermentation, nitrification, methanogenesis, phototrophy, and numerous forms of lithotrophy) could be interpreted in terms of adaptation to chronic energy stress, conditions in which cells are consistently faced with an insufficient supply of cellular energy (high rates of futile ion cycling driven by extreme temperature, acidity or salinity, as well as low rates of cellular energy generation due to limited substrate availability and/or unfavorable thermodynamic conditions; Valentine 2007). From a metabolic perspective in competition between Archaea and Bacteria, the dominance of Archaea is achieved through several successful modes. Halophiles, hyperthermophiles, and thermoacidophiles are groups adapted to cope with a level of energy stress that disables all but few selected bacterial species. As conditions become more moderate, this environmental exclusivity gives way to metabolic exclusivity, which is in the case of methanogens and methane oxidizers. Hence, their dominance is achieved with exclusion of Bacteria by the use of unique catabolic pathways. In moderate environmental conditions, the level of competition between archaea and bacteria intensifies and successful archaea maintain a singularity of catabolism. This allows them to exclude or out-compete bacteria in specific niches with low energy availability. Methanogens, methane oxidizers, and possibly nitrifiers each seemingly display such singularity, with their catabolic emphasis on a single well-defined pathway (Valentine 2007).

Archaeal lipids

The molecular adaptations responsible for archaeal ability to survive and grow in harsh environments have clearly emphasized the key role of membrane lipid components in overcoming the destabilizing conditions encountered in such extreme environments as hot acidic springs and submarine volcanic fields. Since the first archaeal diether polar lipids were discovered in 1960s, more than 100 new lipids have been found in various archaea with great variety of structures.

Polar archaeal lipids are generally composed of a core lipid and a phosphodiester bonded polar head groups or glycosides that are linked to one of the core lipids. Profiles of archaeal lipid core are quite remarkable in their own right even though they are not as diverse as those found in Bacteria and Eukarya. However, four characteristics unique to archaeal lipids compared with bacterial lipids have been identified (Fig. 1) (Koga and Morii 2006). (1) Glycerophosphate backbone of archaeal lipids is composed of *sn*-glycerol-1-phosphate (G-1-P), which is an enantiomer of *sn*-glycerol-3-phosphate (G-3-P) in bacterial phospholipids. Hydrocarbon chains are though bound at the *sn*-2 and -3 positions of the glycerol moiety in archaeal lipids, whereas bacterial lipids have *sn*-1,2-radyl chains. (2) Hydrocarbon chains are bonded to the glycerol moiety exclusively by ether linkages in archaeal polar lipids in contrast to bacterial
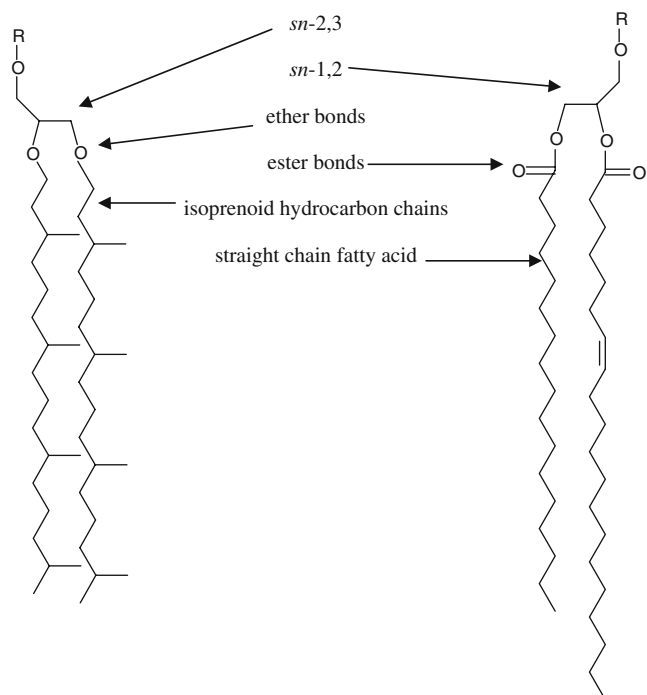

Fig. 1 Typical structures of archaeal phospholipid compared with bacterial phospholipid. Four differences are shown. –R stands for polar head group

lipids, most of which have ester linkages. (3) The hydrocarbon chains of polar lipids in Archaea are highly methyl-branched isoprenoids and isopranoids. Bacterial counterparts are mostly straight chain fatty acids. (4) Significant number of archaeal species contain bipolar lipids with a tetraether core that span through membranes (bolaform amphiphilic), which are rarely found in Bacteria.

Core lipid structure

Basically, major core lipid structures consist of the ubiquitous standard archaeol (2,3-di-O-diphytanil-*sn*-glicerol) consisting mainly of $C_{20,20}$ alkyl chains and/or the standard caldarchaeol (2,2′,3,3′-tetra-O-dibiphytanil-*sn*-diglycerol) with $C_{40,40}$ carbon chains (Fig. 2). Variations to these standard lipid cores include archaeols with increased number of C_5 units or caldarchaeols with cyclopentane rings within the C_{40}-phytanil chains (Fig. 2; Patel and Sprott 1999). Recently, a numerous new core lipid structures have been elucidated (Koga and Morii 2005). Those include isocaldarchaeol from *Methanothermobacter marburgensis* (mixture of regioisomers with antiparallel and parallel arrangements of two glycerol moieties), H-shaped caldarchaeol from *Methanothermus fervidus* (Morii et al. 1998), C_{40} isoprenoid hydrocarbons with cyclo-pentane rings at unusual positions isolated from sedimentary organic matter samples of water column from several marine environments (Hoefs et al. 1997), macrocyclic archaeol with one or two cyclopentane rings in the byphytanediyl chain (Stadnitskaia et al. 2002), alkyl ether type core lipids from *Methanopyrus kandleri* and *Methanococcoides burtonii* (Sprott et al. 1997a), and many more new lipids that are minor modifications of known lipids of archaea (Koga and Morii 2005).

Polar head groups

The polar head groups of archaeal lipids are phospholipids or glycosides that are linked to one of the core lipids. The most common phospholipids are phosphoserin, phosphoinositol, phosphoglycerol, phosphoetanolamin, and many phosphoglyicolipids; among them, the most common carbohydrates found among archaeal lipids are glucose, gulose, mannose, galactose, inositol, and *N*-acetylglucosamine, which can form mono-, di-, or oligosaccharides on one or both sides of caldarchaeol. Phosphoglycolipids with two polar head groups on both sides of the caldarchaeol may have glycerophosphate as the phosphoester moiety on one side and gulose alone or glucose and mannose, which form mono-, di-, or oligosaharides as the sugar moiety on the other side as is in the case of *Thermoplasma acidophilum*

(Shimada et al. 2008). Replacement of one glycerol moiety of the core lipid backbone by a nonitol has also been observed (De Rosa and Gambacorta 1988). The occurrence of unusual carbohydrate ß-D-galactofuranosyl units has been found in methanogens (Gambacorta et al. 1995), but it has not been found in thermoacidophiles (Tables 1 and 2), since the five-member rings in such environments are rapidly hydrolyzed.

Biosynthesis of the polar lipids in thermophilic archea

An excellent review, which focused on in vitro studies of archaeal lipid biosynthesis, has been published by Koga and Morii (2007). Here we present the brief overview of the biosynthesis of the main compounds of archaeal dieter lipids.

1. The hydrocarbon portions of archaeal dieter lipids are exclusively isoprenoids (C_{20} phytanyl, C_{25} sesterter-penyl, or farnesylgeranyl groups). The isoprenoid biosynthetic pathway is divided into two sections. The first half is the synthesis from acetyl-coenzyme A (CoA) to isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), and the second half is the synthesis of polyprenyl diphosphate from the two C_5 units (IPP and DMAPP; Fig. 3). For IPP synthesis, two independent pathways are known: the classical mevalonate (MVA) pathway and more recently discovered MVA-independent (1-deoxy-D-xylulose-5-phosphate pathway; Koga and Morii 2007 and reference cited therein). The archaeal MVA pathway is a mosaic composed of the enzymes common to Archaea and Eucarya, along with the enzymes unique to Archea and Bacteria.

2. G-1-P is the enantiomer of bacterial G-3-P and it is formed from D-glycerolaldehyde-3-phosphate (D-GAP) by three possible reactions. The first possible reaction for G-1-P formation is the reduction of GAP at the position 1. Because D-GAP has the same configuration as G-1-P at the position 2, the simple reduction of its aldehyde group can form G-1-P. The second possibility for the G-1-P formation is the reduction of dihydroxyacetonephosphate at the position 2 catalyzed by G-1-P dehydrogenase, the enzyme responsible for the formation of enantiomeric glycerophosphate backbone structure in archaeal phospholipids. The third possibility for G-1-P formation is the direct phosphorylation of glycerol at the *sn*-1 position by adenosine triphosphate (Koga and Morii 2007 and reference cited therein).

3. The pathway for biosynthesis of the ether-type phospholipids in Archaea is depicted in Fig. 4. The results of in vivo studies suggested that the ether bond is

Fig. 2 Typical archaeal polar lipid cores. **a** Archaeol (C$_{20,20}$), **b** archaeol (C$_{25,25}$), **c** macrocyclic arhaeol, **d** caldarchaeol, **e** isocaldarchaeol, **f** crenarchaeol, **g** H-shaped caldarchaeol containing three cyclopentane rings
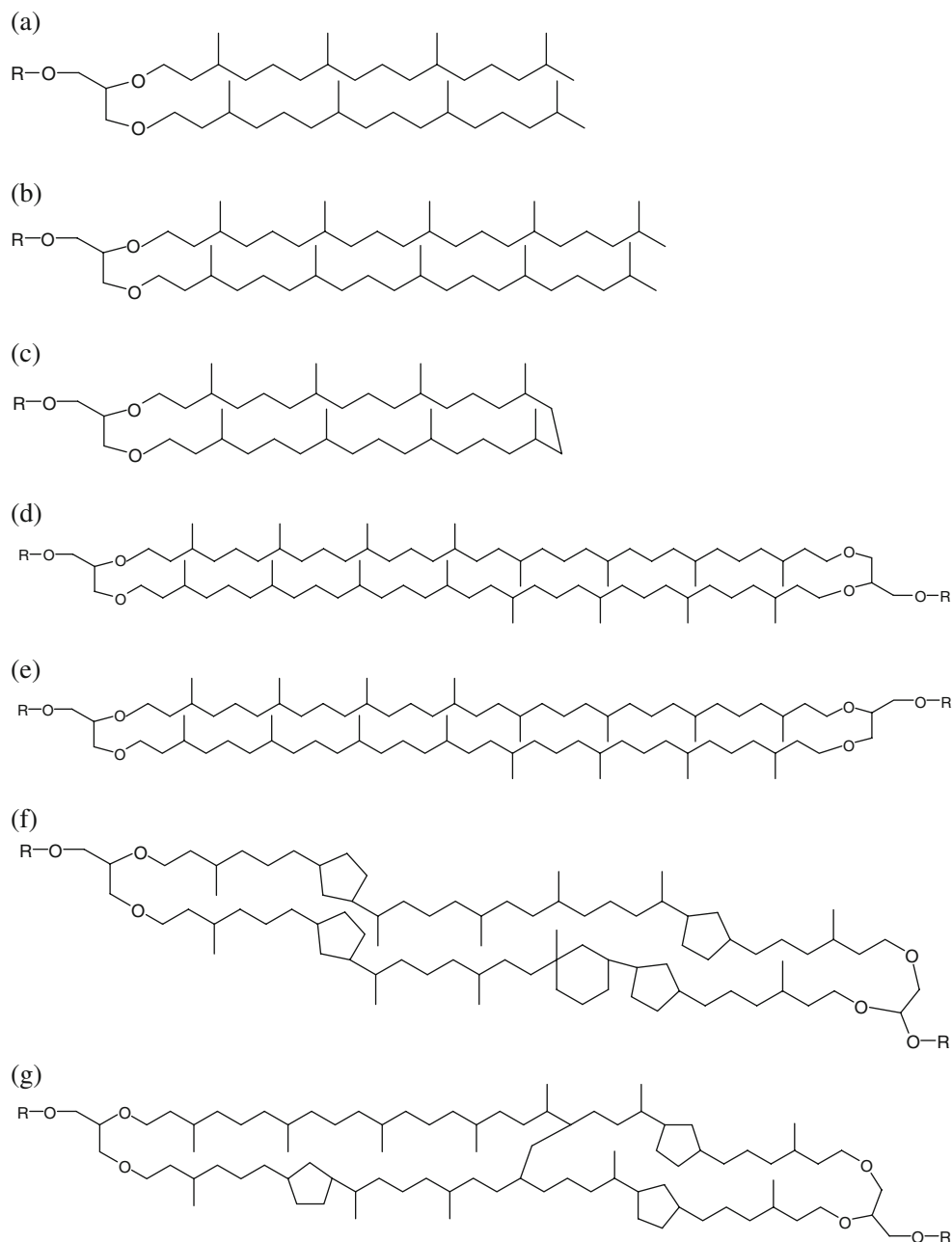
formed from unsaturated prenyl precursors. The bond formation is catalyzed by two prenyl transferases: One is responsible for formation of the first ether bond between the *sn*-3 hydroxyl group of G-1-P and geranylgeranyl diphosphate (GGPP; GGGP synthase), and the other catalyzes the formation of ether bond at the *sn*-2 position to form di-geranylgeranyl-G-1-P (DGGGP or unsaturated archaetidic acid; DGGGP synthase). For details, see the recent review published by Koga and Morii (2007).

4. The final product of thermophilic archaea are fully saturated chains. Hydrogenation or saturation reaction may take place somewhere after attachment of polar head groups in the presence of reductase, which requires nicotinamide adenine dinucleotide (reduced form) as a hydrogen donor via flavin adenine dinucleotide (oxidized). If unsaturated archaetidic acid were saturated, it could not react with CDP-archaeol synthase, which reacts preferentially with unsaturated archaetidic acid.

Physicochemical properties of archaeal lipids

The existence of such a large variety of unusual lipid structures in Archaea raises a question of how these lipids

Table 1 Temperature and pH optima, percentage of tetraethers (TE), type of core lipids, and polar head groups for some hyperthermophilic archaeal isolates with optimal temperature of growth above 80°C and pH above 5.5

Genus and species	pH	T (°C)	TE (%)	Lipid type	Polar heads	References
Archaeoglobus fulgidus	7	83	50	Diether and tetraether with 0–2 cycles	mono- or di-glycosil groups	(Lai et al. 2008)
Palaeococcus ferrophilus	6	83	>80	C_{40} tetraether lipids (>80%) and C_{20}, C_{20} diether lipids (<20%)	/	(Takai et al. 2000)
Pyrococcus woesei	6	101.5	100	Archaetidylinositol	*myo*-L-inositol	(Lanzotti et al. 1989)
P. abyssi	6.8	96	85	15% phytanil glycerol diethers, 73.5 acyclic and 9.5% bipentacyclic C_{40} biphytanyl chains	/	(Erauso et al. 1993)
Thermococcus celer	5.8	88	0	2,3-di-O-phytanyl-*sn*-glycerol ester of phosphatidyl-*myo*-inositol (>80%)	>80% phosphatidyl-*myo*-inositol	(De Rosa et al. 1987)
T. fumicolans	8	85	30	Diphytanil glycerol diether(70%), tetraeters with 0–2 rings (30%)	/	(Godfroy et al. 1996)
T. barophilus	7	85	0	Archaeol	possibly glycolipid	(Marteinsson et al. 1999)
T. kodakarensis	6.5	85	80	Saturated forms of archaeols and caldarchaeols	/	(Matsuno et al. 2009)
Thermococcus hydrotermalis	6.8	79	55	Diphytanil glycerol diether, dibiphytanil diglycerol tetraether, no cyclopentane rings	/	(Lattuati et al. 1998)
Methanococcus jannaschii	6	85	0	Diethers and macrocyclic diethers (macrocyclic archaeol)	Phosphoethanolamine-(1→6)-β-D-glucopyranose, β-D-glucopyranose, and β-D-glucopyranosyl-(1→6)-β-D-glucopyranose	(Comita et al. 1984; Ferrante et al. 1990)
Methanothermus fervidus	6.5	80–85	>91	Caldarchaeol (60%), archaeol (4%), H-shaped C_{80} isoprenoid (31%)	Glucose, *N*-acetylglucosamine, inositol	(Morii et al. 1998; Sprott et al. 1997a)
Methanopyrus kandleri	6.5	97	0	Diethers	1–6 hexose (mannose, glucose, galactose, *N*-acatylglucosamine) residues	Sprott et al. 1997a
Aeropyrum pernix	7	90–95	0	$C_{25,25}$ Diethers	Glucose, inositol	(Morii et al. 1999; Sako et al. 1996)
Desulfurococcus mobilis	5.8	88	100	GDGTs with small amount of phytanil diether compounds and no cyclopntane rings	Galactose, glucose, P-*myo*-inositol	(Lanzotti et al. 1987)
Igniococcus islandicus	5.8	90	50	Acyclic 2,3-di-O-phytanyl-*sn*-glycerol and glycerol-dialkyl glycerol tetraether (GDGT)	/	(Huber et al. 2000)
Pyrolobus fumarii	5.5	106	95	Acyclic glycerol-dialkyl-glycerol-tetraether and traces of 2,3-di-O-phytanyl-*sn*-glycerol (di-ether)	/	(Blöchl et al. 1997)
Thermosphaera aggregans	6.5	85	95	Mainly acyclic and cyclic glycerol diphytanyl tetraethers with one to four pentacyclic rings	/	(Huber et al. 1998)
Pyrobaculum aerophilum	7	100	95	Acyclic and cyclic glycerol diphytanyl glycerol tetraethers with one to four pentacyclic rings	/	(Trincone et al. 1992; Volkl et al. 1993)
P. islandicum	6	100	100	GDGTs with 0–4 cyclopenane rings	*myo*-inositol, β-D-glucose, phosphate group	(Trincone et al. 1992)
P. organotrophum	6	100	100	GDGTs with 0–4 cyclopenane rings	*myo*-inositol, β-D-glucose, phosphate group	(Trincone et al. 1992)
Nanoarchaeum equitans	5.5	90	10	One or two glycosil groups	95.5% mannose and 4.5% glucose	(Jahn et al. 2004)
Ignicoccus sp. strain KIN4/I	5.5	90	20	One or two glycosil groups	95.5% mannose and 4.5% glucose	(Jahn et al. 2004)

Table 1 (continued)

Genus and species	pH	T (°C)	TE (%)	Lipid type	Polar heads	References
Hyperthermus butylicus	7	95–107	>85	Biphytanols with 0–2 cyclopeentane rings (>85%), phytanol (<15%)	/	(Zillig et al. 1990)
Sulfophobococcus zilligii	7.6	87	100	Dibiphytanyldiglicerol tetraethers	*myo*-inositol phosphate, mono- and diglycosyl units	(Hensel et al. 1997)

function in membranes of these organisms. Ether linkages are more stable than ester over a wide range of pH, and the branching methyl groups help to reduce both crystallization (membrane lipids in the liquid crystalline phase at ambient temperature) and membrane permeability (steric hindrance of the methyl side group). The saturated alkyl chains would impair stability toward oxidative degradation particularly in halophiles that are exposed to air and sunlight (Benvegnu et al. 2008). The membranes of methanogens and thermoacidophiles essentially consist of bipolar monolayer structures (Table 2). The high proportions of glycosylated lipids presented in membranes of thermoacidophiles and methanogenes may further stabilize their membrane structures through the interglycosyl headgroup hydrogen bonding. The presence of large sugar heads toward the convex surface of the membrane is likely to promote an asymmetric orientation, thus making the monolayer organization easier. Furthermore, the flux of small molecules and protons through archaeal bipolar tetraether lipid membranes is considerably reduced as the result of the particular physical structure of the lipid monolayer. Finally, the presence of the cyclic diether structures in species isolated from deep-sea hydrothermal vents may be related to the high pressures under which these archaea live.

Thermal stability

The primary and fundamental physiological function of polar lipids is to form a cell membrane, which encloses a cell and makes a permeability barrier for various essential solutes for life. One of the essential general features

Table 2 Temperature and pH optima, percentage of tetraethers (TE), type of core lipids, and polar head groups for some thermoacidophilic archaeal isolates with optimal temperature of growth above 60°C and pH below 5.0.

Genus and species	pH	T (°C)	TE (%)	Lipid type	Polar heads	References
Picrophilus oshimae	0.7	60	100	Caldarchaeol containing 0–5 cyclopentane rings with traces of archaeol	Glucose	(Schleper et al. 1995, 1996)
Metallosphaera sedula	2.8	75	100	Caldarchaeol (50%) and calditoglycerocaldarchaeol (50%)	/	(Itoh et al. 2001)
Sulfolobus solfataricus	3–4.5	87	100	Glycerol dialkyl glycerol tetraethers (GDGTs) with 0–6 rings and a glycerol trialkyl glycerol tetraether (GTGT)	Glycerol	(Ellen et al. 2009)
Thermoplasma acidophilum	1–2	59	100	mostly caldarchaeol	Glycerol	(Uda et al. 2000)
Sulfurisphaera ohkwakuensis	2.0	84	100	Calditoglycerocaldarchaeol and caldarchaeol (8:2)		(Kurosawa et al. 1998)
Thermogymnomonas acidicola	3.0	60	100	Cyclic and acyclic glycerol-bisdiphytanil-glycerol tetraethers		(Itoh et al. 2007)
Caldivirga maquilingensis	4	85	100	Tetraether core lipids and trace amounts of diether core lipids		(Itoh et al. 1999)
Thermocladium modestius	4	75	100	Glycerol-bisdiphytanyl-glycerol tetraethers		(Itoh et al. 1998)
Caldisphaera lagunensis	3.5–4.5	70–78	100	Cyclic and acyclic tetraethers		(Itoh et al. 2003)
Aciduliprofundum boonei	4.5	70	100	Glycerol dibiphytanil glycerol tetraethers (GDGT) containing 0–4 cyclopentane rings with presence of H-shaped GDGTs	Phospho-glycerol	(Schleper et al. 1995; Reysenbach et al. 2006)

Fig. 3 MVA pathway for synthesis of isopentenyl diposphate (IPP) and dimethylallyl diposphatehyl (DMAPP) and further formation of geranylgeranyl-PP (GGPP) (Koga and Morii 2007)
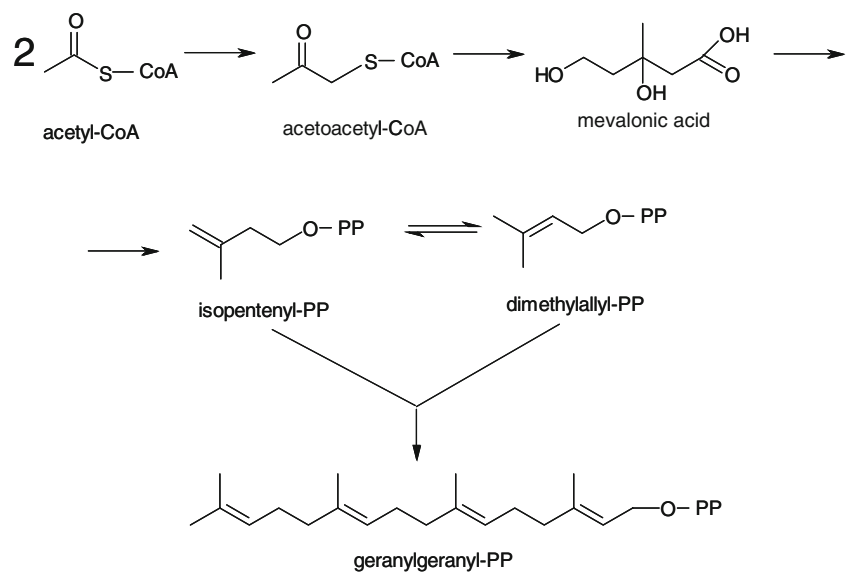
required for lipid membrane to fulfill biological functions is that they are in the liquid crystalline phase. Archaeal lipid membranes have, in general, much lower phase transition temperature than fatty acyl ester lipids (Yamauchi et al. 1993). While membranes made of fatty acyl ester lipids are in the gel phase or in the liquid crystalline phase depending mostly on their fatty acid composition, archaeol- and caldarchaeol-based polar lipid membranes of archaea are assumed to be in the liquid crystalline phase at a wide temperature range of 0–100°C. Recently, it has been shown in vivo by applying the electron paramagnetic resonance and fluorescence emission spectroscopy that the membrane fluidity of hyperthermophilic archaeon *Aeropyrum pernix* consisted solely of 2,3-di-*O*-sesterterpanyl-*sn*-glycerol core lipid (C$_{25,25}$-archaeol; Morii et al. 1999), gradually increases with the temperature up to 65°C, the lower temperature limit of *A. pernix* growth (Ulrih et al. 2007). Similar results were observed for in vitro study on the total polar lipids (TPL) isolated from thermophilic archaeon *Sulfolobus acidocaldarius*, which become fluid at temperature close to minimum growth temperature (~50°C) of thermoacidophilic archaeon (Khan and Chong 2000). Additionally, differential dynamic calorimetry (DSC) and pressure perturbation calorimetry (PPC) were used to characterize thermal phase transition, membrane packing, and volumetric properties of multilamellar vesicles prepared from TPL isolated from *S. acidocaldarius* (Chong et al. 2005). In the temperature range at ~47–50°C and ~69°C, two phase transitions were detected by DSC, which involve very small volume changes, the first one is ascribed to lamellar-to-lamellar phase transition, while the second one with significant enthalpy changed to lamellar-to-cubic phase transition. These phase transitions are followed with very low volume changes, indicating that the polar

headgroup region of TPL may still be rigid and tightly packed through hydrogen-bond network at elevated temperatures.

Permeability

The transport of small molecules across lipid bilayers is a fundamental biological process. Given the extreme environmental conditions in which these archaea thrive, their membranes have to fulfill two conditions required for a biological membrane, liquid crystalline phase and low permeability, with almost the same composition in a wide temperature range. It has been observed that archaeal cultures grown at different conditions exhibit different lipid membrane ratios as is the case of *Archaeoglobus fulgidus*, where change in tetraether/diether lipid ratio and number of pentacycles in the hydrocarbon chains was observed between cultures grown at 70°C or 89°C, respectively (Lai et al. 2008). Here, we focus on hyperthermophiles and acidothermophiles (Tables 1 and 2). The membrane of acidothermophiles is composed of 100% tetraether lipids, while for (neutro)-hyperthermophiles, the ratios between tetraether and diether lipids is strain dependent (Tables 1 and 2). In vitro study using the liposomes (archeosomes) made from caldarchaeol lipids of *S. acidocaldarius*, which has an optimal growth temperature of 85°C at pH 2.0, at temperatures below 40°C, no proton permeability was observed (Elferink et al. 1994). In the similar study of the major polar lipids from *S. acidocaldarius*, it was shown that membrane surface charge was responsible for low 5,6-carboxylfluorescence permeability but not for proton permeability (Elferink et al. 1994). More examples are listed in following paragraphs describing archaeosomes.
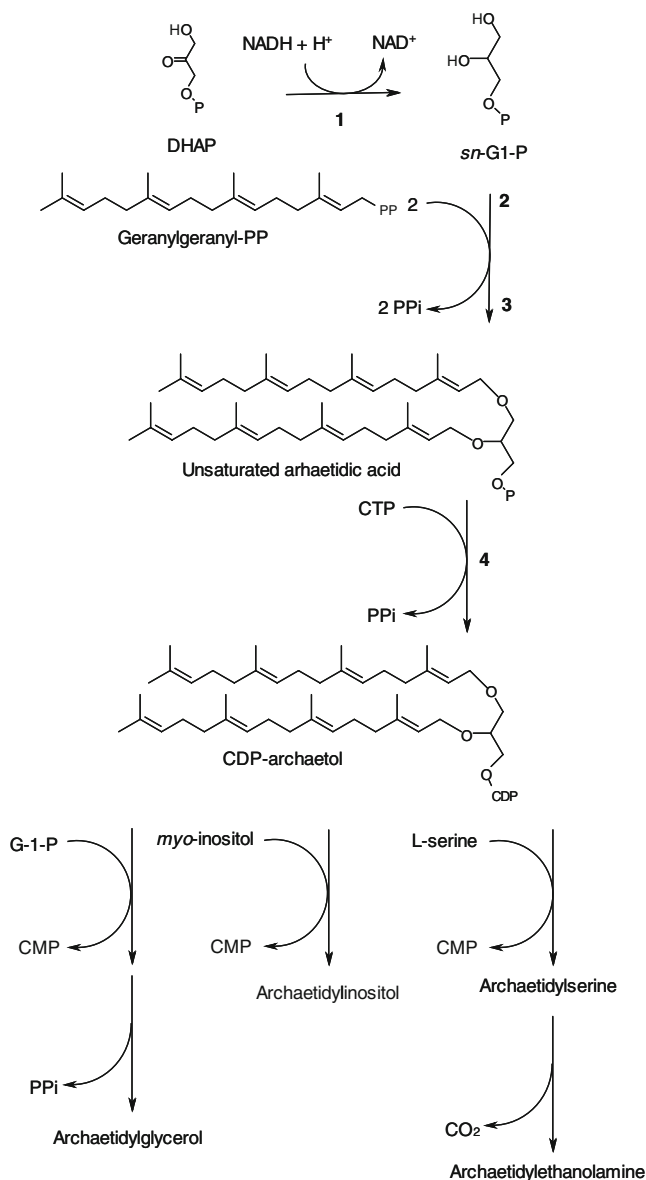
Fig. 4 The proposed biosynthetic pathway for phospholipids synthesis in archaea (Koga and Morii 2007). Enzymes confirmed by in vitro experiments are as follows: *1* G-1-P dehydrogenase; *2* GGGP synthase; *3* DGGGP synthase; *4* CDP-archaeol synthase

It is known that the structural differences between lipids affect solute permeation through the membranes. In the polar headgroup regions of bipolar tetraether liposomes, there is an extensive network of hydrogen bonds, which should generate a high electrical dipole potential, thus hindering solute permeability through membranes. Unlike non-archaeal monopolar diester/diether lipids, archaeal bipolar tetraether lipids do not have the hydrocarbon terminal methyl groups; instead, the biphytanyl hydrocarbon chains are linked covalently from one polar end to the other, lacking the midplace spacing. Furthermore, there are cyclopentane rings in the phytanyl chains, which provide

additional rigidity to the membrane. Because of the stiffness in the molecule, bipolar tetraether lipids should exhibit rather limited *gauche-trans* isomerization in their hydrocarbon chains. Conceivably, solutes cannot easily move in the hydrocarbon region of the bipolar membrane via "hopping." From the viewpoints of penetration and "hopping" solute permeation across archaeal bipolar tetraether lipid membranes should be less than that for archaeal dieter lipids and non-archaeal monopolar diester or dieter lipid membrane (Gliozzi et al. 2002 and reference cited therein).

Artificial archaeal lipid membranes—archaeosomes

Archaeal lipids were shown to be an excellent source for the formation of liposomes (archaeosomes) with remarkable thermostability and tightness against solute leakage (Gambacorta et al. 1995). Ether lipids are also resistant to enzymatic degradation by phospholipases, and archaeal liposomes are exceptionally stable; they do not fuse or aggregate during storage at 4°C over a period of 4 months (De Rosa 1996). Membrane stability can be conveniently monitored by determining the release of fluorescent dyes originally trapped in the intravesicular compartment of the liposomes. In particular, several studies have focused on investigation of membranes made solely from bipolar lipid fractions. Different physicochemical properties (structure, dynamics, and polymorphism and thermal and mechanical stability) of bipolar lipid fractions extracted from several archaeal species have been investigated. However, there are different views on the specific structural factors responsible for the high thermostability. Chang and Elferink (Chang 1994; Elferink et al. 1994) suggested that the high thermostability is not due to the phytanyl chain alone, in contrast to the perception of Yamauchi and his coworkers (Yamauchi et al. 1993).

Arakawa et al. (1999) investigated the polymorphism and physicochemical properties of the macrocyclic lipids by synthetic 72-membered macrocyclic tetraether lipids. DSC, ^{31}P NMR and electron microscopy analysis studied the physicochemical features of diphospholipids. The cyclic tetraetherlipids appeared to show lower phase transition temperature (T_c). Fluorescence studies have shown that the passive proton permeability in bipolar tetraether liposomes isolated from *S. acidocaldarius* is lower and less temperature sensitive than that in liposomes composed of monopolar diester lipids, although the permeability increases with temperature in all liposomes. It has been proposed that low proton permeability is due to the chemical structure of tetraether lipids and their monolayer organization, especially the cyclopentane rings and the network of hydrogen bonds between the sugar residues

exposed at the outer face of the membrane (Chong et al. 2003, Mathai et al. 2001).

In general, it was shown that, at a given temperature, the bolaform lipid chains are more ordered and less flexible than in conventional bilayer membranes. Only at elevated temperatures (80°C) does the flexibility of the chain environment in tetraether lipid assemblies approach that of fluid bilayer membranes (Bartucci et al. 2005). By examination of water, solute (urea and glycerol), proton, and ammonia permeability of archaeosome, it was shown that macrocyclic archaeol and caldarchaeol lipids reduced the water, ammonia, urea, and glycerol permeability significantly (6–120-fold) compared to dipalmitoylphosphatidylcholine (DPPC) liposomes (Mathai et al. 2001). The presence of an ether bond reduced the apparent proton permeability for threefold compared to DPPC liposomes. The presence of the ether bond and phytanyl chains did not significantly affect these permeabilities. The presence of macrocyclic archaeol and caldarchaeol structure further reduced apparent proton permeability by 10–17-fold (Mathai et al. 2001). Komatsu and Chong have suggested that the low leakage rates of anionic probe at neutral pH are due to the negative charges of polar tetraether lipid membrane surface in addition to the tight and rigid lipid packing (Komatsu and Chong 1998). Some studies have shown that the leakage rate of entrapped dyes increases with decreasing the content of tetraether lipids and increases in the presence of fusogenic agents such as calcium ions and polyethylene glycol. These observations and many others suggest that the degree of hydration and conformation of the lipid polar headgroup are crucial for the tetraether membrane integrity. Furthermore, the release of entrapped dyes in bipolar tetraether liposomes is less temperature sensitive than that in non-archaeal monopolar diester liposomes (Mathai et al. 2001), suggesting a low thermal expansibility for tetraether liposomes (Chong et al. 2003). This general property is important for thermophiles, since they may experience temperature fluctuations in their native environment.

Biotechnological applications of archeosomes

Both in vitro and in vivo studies indicate that archaeosomes are safe. Their pH and thermal stability, tissue distribution profiles, as well as the adjuvant activity of archaeosome formulations indicate that they may offer a superior alternative for several biotechnological applications, including delivery systems for drugs, genes, or cancer imaging agents (Table 3).

As an ideal vector in drug and gene delivery should be highly efficient in delivering the drug in a target-specific manner (various molecules can be incorporated into archaeosomes or associated with the vesicles to target-specific tissues), stable in vitro as well as in vivo, nontoxic and non-immunogenic, archaeosomes delivery systems answer most of these conditions. The two important observations are (1) archaeosomes promote strong humoral and cell-mediated (Th1/Th2) immune reactions to soluble-entrapped proteins. More importantly, archaeosomes induce CD8+ cytotoxic T lymphocyte responses (critical for protection against cancer and intracellular infections) to entrapped proteins. Thus, archaeosomes are self-adjuvanting delivery vesicles (Sprott et al. 1997b; Sprott et al. 1999) and (2) better immunization with archaeosomes is achieved (Sprott et al. 2003). Recently, two excellent reviews were published covering the archaeosome adjuvants from immunological capabilities to mechanism(s) of action (Krishnan and Sprott 2008), and a synthetic route for the preparation of symmetrical and unsymmetrical archaeal tetraether-like analogs has been described for use in these purposes (Brard et al. 2007). Archaeal lipids have also been proposed as

Table 3 Biotechnological applications of tetraether archaeal lipids

Application	Reference
Taxonomic markers	(Gambacorta et al. 1995; Koga and Morii 2005)
Lubrication	(Chang 1992)
Preparation of membranes for separation processes	(Bauer et al. 1983)
Antigen delivery systems	(Patel and Sprott 1999)
Protein delivery system	(Sprott et al. 1997b, 1999)
Immobilization of the molecule	(Berzina et al. 1995, 1997)
Imunization/vaccine adjuvant	(Sprott et al. 2003; Krishnan and Sprott 2008)
Food industry-	(Mozafari et al. 2006)
Bioelectronics	(De Rosa et al. 1994)
Ultratinlayers for biosensors	(Meister and Blume 2007)
Biomimetric materials (film deposition on solid surface application in filter permeability)	(Muller et al. 2006)
Synthetic preparation of archaeal lipid analogs	(Brard et al. 2007)

monomers for bioelectronics for several reasons, the most important being that their bipolar tetraether structure offers novel opportunities for protein–lipid interactions, which are of interest for the assembly of electronic devices based on redox proteins or enzymes (De Rosa et al. 1994). Some other applications include use of archaeal lipids as taxonomic markers (Gambacorta et al. 1995) and as novel lubricants for engines, since they are chemically stable at high temperatures, lubricate, either with or without a carrier lubricant, and provide a friction coefficient of less than 0.1 (Chang 1992).

Conclusions

The organism's response to extremely low pH is the opposite of that to high temperature. The literature data indicate that the presence of bipolar tetraether lipids is not necessary for thermal adaptation. A significant number of hyperthermophilic archaea does not contain tetraether lipids at all (Tables 1 and 2). Some neutrophilic hyperthermophiles contain $C_{25,25}$ diether lipids. The chain length of the C_{25}-isoprenoid hydrocarbon is 20% longer than those of the C_{20}-isoprenoid and C_{18} straight-chain fatty acids. Therefore, with it composed of only the $C_{25,25}$-archaeol-based lipids, the thickness of the membrane of *A. pernix* is assumed to be 20% greater than the membranes of the $C_{20,20}$-archaeol-based lipids of other archaea (Morii et al. 1999). Furthermore, the presence of variety of polar head groups in an archaeal membrane is not necessary for adaptation to thermal environments. The adaptation of thermophiles to acidic environments requires the presence of tetraether polar lipids in addition to polar heads to maintain the low permeability of the membrane (Tables 1 and 2). The higher temperature adaptation might or might not require the higher number of cyclopentane rings compared to low pH. The number of sugar lipids units of glyco(phospho)lipids increase under high temperature and low pH conditions. The sugar moiety of lipids may interact with each other via hydrogen bonding. The network with increased sugar units of polar lipids at low pHs may have decreased membrane fluidity. The number of cyclopentane rings may have decreased to increase membrane fluidity at low pH and to counteract the effect of increased amount of sugar moieties and vice versa at higher temperature. All this characteristics of unique lipid structures and properties of archaeal lipids are important when development of novel biotechnological processes/products or their application is considered. It is obvious that application will be further developed on the basis of our understanding of archaeal lipids functioning in natural environment, which will open new possibilities in new technologies such as biotechnology, nanotechnology, and also in farma and nutria applications.

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