



F 3.2-0004

THE EVOLUTION OF LIPIDS

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ABSTRACT

Living organisms on the Earth which are divided into three major domains - Archaea, Bacteria, and Eucarya, probably came from a common ancestral cell. Because there are many thermophilic microorganisms near the root of the universal phylogenetic tree, the common ancestral cell should be considered to be a thermophilic microorganism. The existence of a cell is necessary for the living organisms; the cell membrane is the essential structural component of a cell, so its amphiphilic property is vital for the molecule of lipids for cell membranes. Tetraether type glycerophospholipids with C₄₀ isoprenoid chains are major membrane lipids widely distributed in archaeal cells. Cyclization number of C₄₀ isoprenoid chains in thermophilic archaea influences the fluidity of lipids whereas the number of carbons and degree of unsaturation in fatty acids do so in bacteria and eucarya. In addition to the cyclization of the tetraether lipids, covalent bonding of two C₄₀ isoprenoid chains was found in hyperthermophiles. These characteristic structures of the lipids seem to contribute to their fundamental physiological roles in hyperthermophiles. Stereochemical differences between G-1-P archaeal lipids and G-3-P bacterial and eucaryal lipids might have occurred by the function of some proteins long after the first cell was developed by the reactions of small organic molecules. We propose that the structure of lipids of the common ancestral cell may have been similar to those of hyperthermophilic archaea.

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INTRODUCTION

Almost 1.5 million species on the Earth probably came from a common ancestral cell. All these living organisms have common characteristics such as a structural unit like a cell and genetic information carried by DNA and expressed as protein via RNA. They are divided into three major domains - Archaea, Bacteria, and Eucarya (Woese et al., 1990) as shown in Figure 1. Because there are many thermophilic microorganisms near the root of the universal phylogenetic tree based on the small subunit rRNA gene sequences, the common ancestral cell should be considered to be a thermophilic microorganism. Before biological evolution occurred, biological materials had been synthesized through chemical evolution. Existing organisms carry the records of chemical evolution and the evolution of life from the birth of the universe.

About 85 % of human genome sequence was opened to the public on June 26, 2000 (<http://www.ncbi.nih.gov/genome/seq/HsHome.shtml>). The genomes of 29 species including 6 archaea and 23 bacteria also have been completed and presented in public databases as of July 2000. Next, interest will be focused on understanding the function of these gene products. The number of sequences in the archaeal strains is still insufficient to be used to compare the sequences in the eucaryotic and bacterial domains;

however, the phylogenetic relationships of the genes among living organisms and the evolutionary understanding of the genes will be uncovered in the coming century.

The existence of a cell is necessary for the transfer of genetic information from parents to daughter for conservation of the species. The cell membrane is the essential structural component of a cell. The most fundamental function of the membranes of a cell is to separate an inner water layer from an outer water layer to form selective-permeability barriers. Many amphiphilic lipids can form monolayers at the air-water or oil-water interface. In the case of the amphiphilic molecules with one hydrocarbon tail, they can also form micelles in which hydrophobic tails collect in a sphere of hydrocarbon and polar heads project into the surrounding water. On the other hand, amphiphilic molecules with two hydrocarbon tails can form lipid bilayers. Glycerol and sphingosine are the most suitable molecule to form the membrane lipids which have two parallel or antiparallel hydrophobic tails and one polar head. The major structures of the cell membrane are usually lipid bilayers which are constructed with complex lipids such as glycerol- or sphingolipids. The polar heads of these lipids are placed on outside and inside of the membranes.

We are interested in the structure of lipids of a common ancestral cell, so we have been investigating the structure of thermophilic archaeal lipids for two decades. In this paper, we first present the summary of our results focused on the unique properties of their simplicity and stability, then discuss the evolution of lipid.

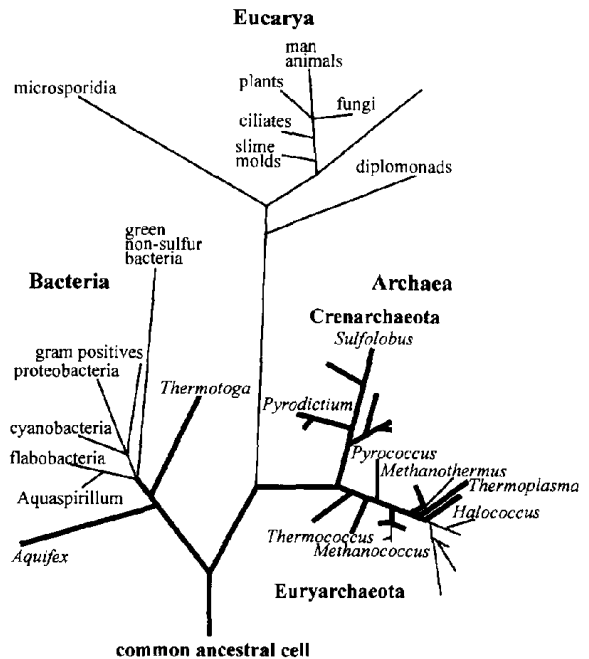


Fig. 1. Universal phylogenetic tree based on small subunit rRNA gene sequences (Modified from Woese *et al.* 1990). Bold lines lead to hyperthermophiles (Modified from Baross *et al.* 1996)

THE DIFFERENCES BETWEEN ARCHAEOAL LIPIDS AND BACTERIAL AND EUCARYAL LIPIDS

Figure 2 shows the differences between the structure of archaeal lipids and bacterial and eucaryal lipids. Glycerophospholipids are major components of bacterial and eucaryal membranes. The nonpolar tails of the lipids of bacteria and eucarya are long-chain fatty acid molecules attached to a polar head group such as *sn*-glycerol-3-phosphate by ester bonds. The hydrocarbon chains of a fatty acyl group contain 12 to 24 carbon atoms. About half of the fatty acyl groups are saturated and the other half of them are unsaturated or polyunsaturated. The degree of saturation of the hydrocarbon chains of a fatty acid and the length of the chain itself influence the melting point of the fatty acid. A variety of glycerophospholipids are derived from many ionic and polar substituents such as choline, ethanolamine, serine, glycerol, phosphatidylglycerol or *myo*-inositol attached to the phosphate group of glycerophospholipids (Figure 2(B)). On the other hand, archaeal lipids are composed of isoprenoid alcohols attached to a *sn*-glycerol-1-phosphate by ether bonds (Figure 2(A)). Thus, the stereochemistry of the glycerophosphate backbone of phospholipids of archaea and of bacteria and eucarya are entirely opposite (Kates, 1972, Langworthy and Pond 1986, and Koga *et al.* 1998). Distribution of lipid species and major lipid properties of archaea, bacteria and eucarya are

compared in Table 1. Besides so many biological functions, steroids have a role to play in reinforcement of the membrane. Derivatives of the triterpene family are phylogenetic precursors of sterols and are widely distributed in procaryotes (Rohmer et al. 1979, and Ourisson and Nakatani 1994).

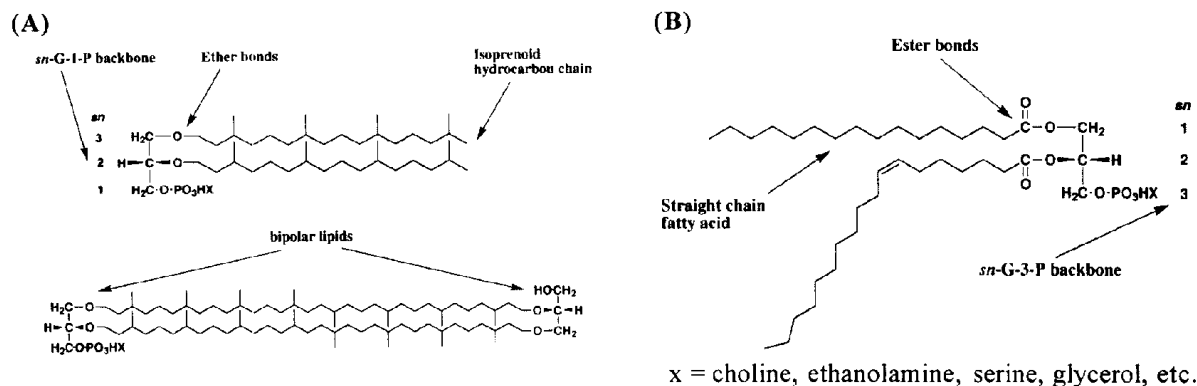


Fig. 2. Structures of archaeal polar lipids (A) and bacterial and eucaryal polar lipids(B).

Table 1. Major lipid properties of archaea, bacteria, and eucarya

	Archaea	Bacteria	Eucarya
Glycerolipids	+	+	+
Hydrocarbon chain	Isoprenoid	Fatty acid	Fatty acid
Size of hydrocarbon	C ₁₅₋₂₅ / C ₄₀	C ₁₂₋₂₄	C ₁₂₋₂₄
Hydrocarbon bonding type	Ether	Ester	Ester
Position in glycerol	<i>sn</i> -2, 3	<i>sn</i> -1, 2	<i>sn</i> -1, 2
Phospholipids	+	+	+
Glycolipids	+	+	+
Phosphoglycolipids	+	+	+
Sulfoglycolipids	+	-	+
Phosphosulfoglycolipids	+	-	-
Sulfolipids	-	- / (+)	+
Sphingolipids	-	- / (+)	+
Triterpen Family	+	+	+
Steroids	-	-	+

+, present; -, absent; - / (+), present in some genus or species

Isoprenoid chains in archaeal lipids vary in size from C₁₅ to C₄₀. In the case of C₁₅ to C₂₅ or cyclic C₄₀ used to form a membrane, a lipid bilayer is present (Kates 1972). A C₄₀ isoprenoid chain can contain from zero to a maximum of four cyclopentane rings (De Rosa et al., 1980). Also two C₄₀ isoprenoid chains are combined around the center of the chains by a covalent bond. This is called C₄₀ H-form (Morii et al., 1998 and Sugai et al., 2000). These isoprenoid chains are distributed in a specific manner in three major classes of archaeal strains. Halophilic lipids are mainly composed of C₂₀ and some of C₂₅ isoprenoid alcohols to form the diether bilayers as shown in Figure 3(a) (Kates, 1992). Methanogenic lipids contain C₂₀ and C₄₀ isoprenoid alcohols (Koga et al., 1993). When major thermophilic lipids are composed of C₄₀ isoprenoid alcohols, a bipolar tetraether monolayer can be formed (Langworthy and Pond 1986). Variations of the typical major lipid structures of the strains of three groups of archaea are shown in Figure 3(A).

Sterols contribute to increasing the rigidity and amphiphilic character of the eucaryal membranes, whereas they are normally absent in bacterial and archaeal membranes. Ourisson and Nakatani (1994) reported that derivatives of one triterpene family (the hopanes) are widely distributed in bacteria, acting as

reinforcers in their membranes, the same role as sterols in eucarya. These researchers also suggest that these molecules seem to be the evolutionary precursors of archaeal lipids. In the case of thermophilic archaea, C_{40} tetraethers figure in an excellent manner for obtaining stability (Rohmer *et al.* 1979). Hypothetical models of membranes of three groups of archaea are shown in Figure 3(B).

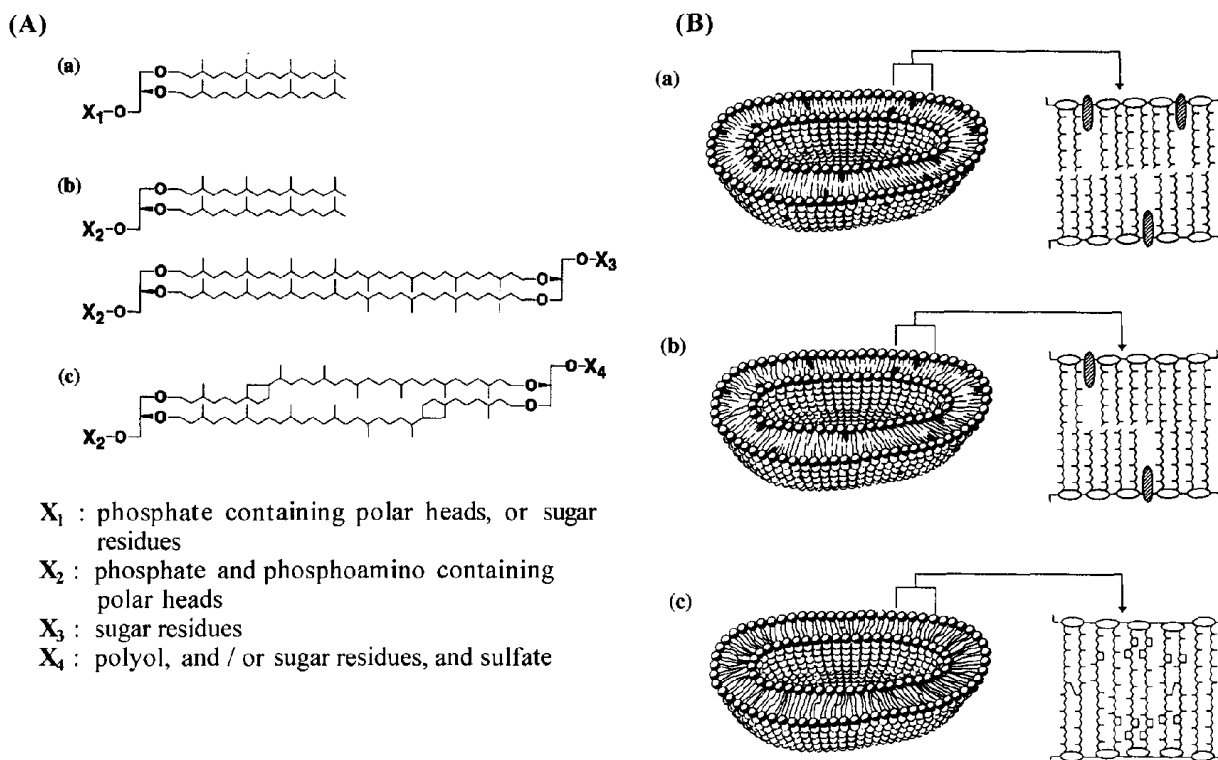


Fig. 3. Typical major lipid structures (A) and schematic diagrams (B) of the membranes of three groups of archaea; (a) halophilic archaea, (b) methanogenic archaea, and (c) thermophilic archaea.

THE STRUCTURES OF CORE LIPIDS OF THE THERMOPHILIC ARCHAEAL STRAINS

We have analysed and compared the structures of core lipids of three strains of thermophilic archaea, *Thermoplasma acidophilum* ATCC27658, *Sulfolobus acidocaldarius* N-8, and *Pyrococcus horikoshii* OT3. General properties and optimum growth conditions of the strains are shown in Table 2. *T. acidophilum* is a wall-less euryarchaeon that grows optimally around 60 °C in an aerobic condition. *S. acidocaldarius* is a well known cren archaeon which grows optimally around 75 °C in an aerobic condition. *P. horikoshii* isolated from hydrothermal vent is an anaerobic hyperthermophilic euryarchaeon. It thrives optimally around 95 °C.

The structures of the core lipids of thermophilic archaea are as follows: archaeol is a diether core lipid with two C_{15} to C_{25} isoprenoid chains, caldarchaeol and calditocaldarchaeol are major tetraether core lipids, caldarchaeol trialkyl type is also a tetraether core lipid and may be derived from an intermediary metabolite from two diether lipids. The cyclization number of isoprenoid chains vary up to four in one chain. Each strain contains specific compositions of these tetraether core lipids. For example, *S. acidocaldarius*

contains calditocaldarchaeol at about 70 % and caldarchaeol at about 20 % of the total lipids (Sugai et al., 1995). H-form caldarchaeol was found only in *P.horikoshii* with contents from 15 to 30 % depending on the growth conditions (Sugai et al., 2000).

Table 2. General properties and optimum growth conditions of thermophilic archaea

	<i>T.acidophilum</i>	<i>S.acidocaldarius</i>	<i>P.horikoshii</i>
Subdomain	Euryarchaeota	Crenarchaeota	Euryarchaeota
Growth temperature, °C	45-63	55-85	80-102
(Optimum, °C)	(60)	(70-75)	(95)
Growth pH (Optimum)	2 (2)	2-3 (2-3)	6-8 (7)
O ₂ requirement	+	+	-
G+C contents, %	46	37	42
Cell wall	-	+	+
Type of major lipids	Tetraether	Tetraether	Tetraether

CYCLIZATION NUMBER OF C₄₀ ISOPRENOID CHAINS DEPENDS ON THE GROWTH TEMPERATURE

The cyclization number of C₄₀ isoprenoid chains changed significantly depending on the growth temperature of the strains of *T.acidophilum* (Uda et al., 2001) and *S.acidocaldarius* (De Rosa et al., 1980) as shown in Table 3. The optimum growth temperature of *P.horikoshii* is 10 °C higher than the maximum growth temperature of *S.acidocaldarius*; however, the cyclization number of core lipids in this strain was smaller than our expectation (Sugai et al., 2000). It is known that the number of carbons and degree of unsaturations in fatty acids influence fluidity of lipids in bacteria and eucarya. Archaeal lipids contain cyclopentane rings instead of double bonds for changing fluidity to adapt to the growth conditions of a strain. The rigid structure of cyclopentane rings in the isoprenoid chains makes the membrane less fluid. In addition to cyclization of the tetraether lipids, covalent bonding of two chains may contribute to their fundamental physiological roles in hyperthermophiles. These results (Table 3) show that the hyperthermophilic archaeal tetraether lipids seem to be the simplest and most stable membrane lipids in existing living organisms.

Table 3. Average cyclization number of C₄₀ isoprenoid chains from the cells harvested at different growth temperatures (°C)

Growth condition of the cells	Average cyclization number of C ₄₀ isoprenoid chains		
	<i>T.acidophilum</i>	<i>S.acidocaldarius</i>	<i>P.horikoshii</i>
High temperature	2.0 (60 °C)	2.4 (82 °C)	0.2 (103 °C)
Optimum temperature	2.0 (60 °C)	1.8 (75 °C)	0.2 (98 °C)
Low temperature	1.6 (40 °C)	1.7 (65 °C)	0.0 (82 °C)

THE EVOLUTION OF LIPIDS

Before the evolution of life occurred, biological materials had been synthesized through chemical evolution. In the case of lipids for cell membranes, amphiphilic property is necessary for the molecule. Glycerol could be selected as the most suitable molecule which can hold two hydrophobic long chains and

one polar head. Isoprenoid chains are synthesized by fewer steps than fatty acid chains including almost the same number of carbons. Tetraether monolayer lipids with a covalent bond in the center of a molecule seems to be the simplest and most stable. A common ancestral cell membrane might have been constructed with molecules such as H-form caldarchaeol.

It is interesting that not only the G-1-P dehydrogenase gene was found and the enzyme was purified from *Methanobacterium thermoautotrophicum* (Koga *et al.*, 1998) but also the G-3-P dehydrogenase gene ortholog was found and a little activity was detected in the same strain (Nishihara *et al.* 1999). Stereochemical differences between G-1-P archaeal lipids and G-3-P bacterial and eucaryal lipids might have occurred by the function of some proteins long after the first cell was developed by the reactions of small organic molecules. From six archaeal whole genome analyses, less than one third of the open reading frames of the genome sequences are expected for their functions up to now. Probably, we will be able to discuss the evolution of lipids from an ancestral cell more definitively after functional analysis of archaeal genomes has progressed.

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