Archaeal Lipids: Biosynthesis

12.458 Molecular Biogeochemistry

9/20/2006

Topics

Review, background:

- Isoprenoids
- Squalene
- Characteristics of Archaeal lipids

Biosynthesis of diether lipids of Archaea

- Chen et al.

Biosynthesis of tetraether lipids

- Kon et al.
- Eguchi et al.

Isoprenoids

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Squalene

Formed through condensation of 6 isoprene units

Farnesane an intermediate

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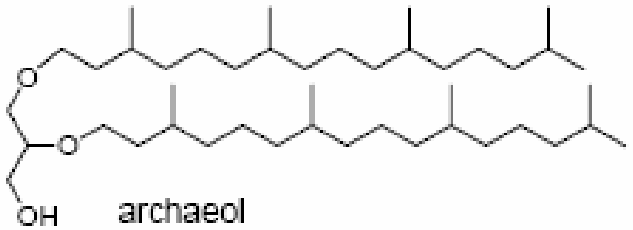
http://www-personal.une.edu.au/~sglover/CHEM303%20Chapter%201%20HTML/img048.gif

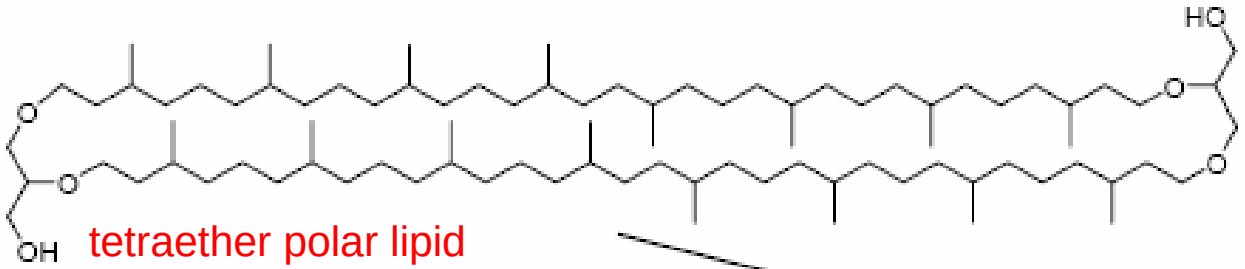
Polar Lipids of Archaea



archaeol

phytane

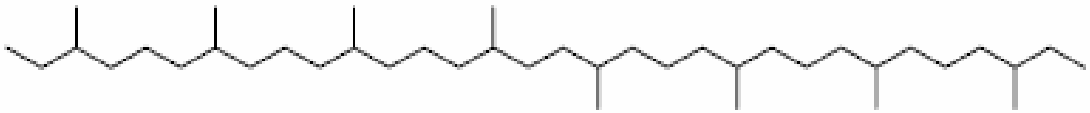
diether polar lipid



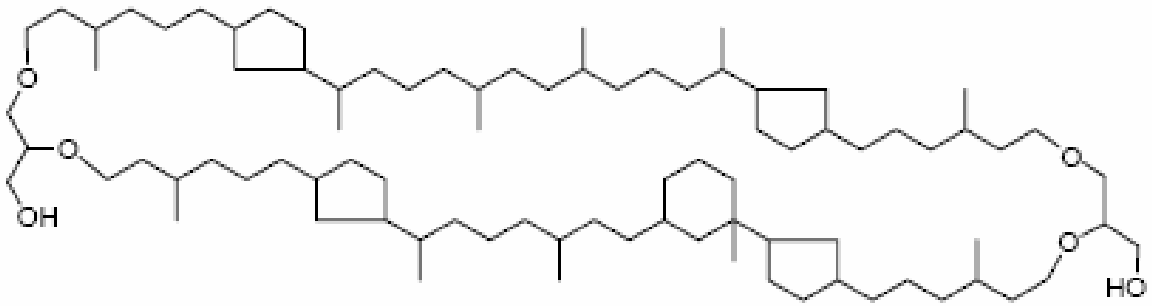
caldarchaeol

tetraether polar lipid

macrocyclic



biphytane



chrenarchaeol

(after Roger)

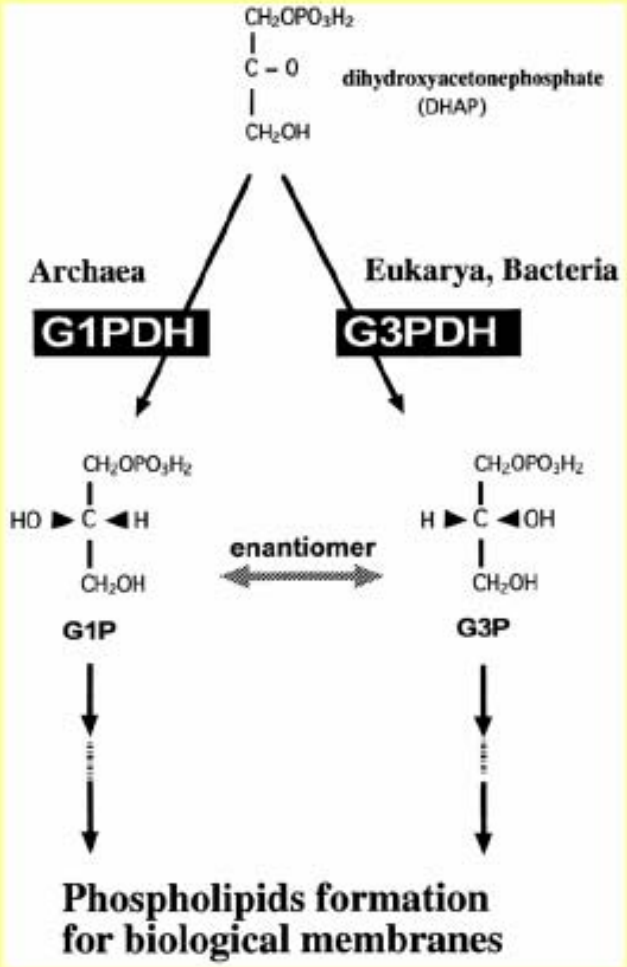
Characteristics of lipids of Archaea

-Ether linkages between isoprenoid chains and glycerol

- Glycerol stereochemistry opposite of that of Bacteria, Eukarya

Glycerol Stereochemistry ?

sn-glycerol-1-phosphate dehydrogenase (G1PDH)



sn-glycerol-3-phosphate dehydrogenase (G3PDH)

(Roger)

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(*S*)-Geranylgeranylglyceryl Phosphate Synthase

PURIFICATION AND CHARACTERIZATION OF THE FIRST PATHWAY-SPECIFIC ENZYME IN ARCHAEBACTERIAL MEMBRANE LIPID BIOSYNTHESIS*

(Received for publication, April 7, 1993, and in revised form, June 24, 1993)

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Previous work

Labeling studies: Archaeal isoprenoids are assembled from acetate by a mevalonate pathway (De Rosa et al.)

Few enzymes in pathway reported:

hydroxymethylglutaryl-CoA reductase in *Halobacterium halobium* (Cabrera et al.)

isopentenyl diphosphate isomerase in *Methanobacterium thermoautotrophicum* (Zhang and Poulter)

2 enzymes required for synthesis of core membrane diethers in *M. thermoautotrophicum* (Zhang and Poulter)

→ **Scheme I**

Scheme I: 2 enzymes required

GGGP Synthase
geranylgeranyl phosphate

Catalyzes alkylation of GP

DGGPP Synthase

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- GGPP purified from *Methanobacterium thermoautotrophicum* through chromatography, electropheresis

Characteristics of the first GGPP enzyme

-Enzyme activity requires divalent metal

Mg^{2+} Mn^{2+} Zn^{2+} (not Ca^{2+})

-Maximal activity at 65°C, pH 6.0-7.5

Mechanism for alkylation of (S)-GP by GGPP, a prenyltransferase

-Related to electrophilic reactions of farnesyl diphosphate synthase, dimethylallyl tryptophan synthase

-Catalyzes rupture of C-O bond in diphosphate-isoprene linkage

-Generates electrophilic allylic carbocations that alkylate prenyl acceptors

GGPP synthase catalyzes the first committed step in the biosynthesis of diether core lipids in Archaea.

JOURNAL OF BACTERIOLOGY, Mar. 2002, p. 1395–1401 Vol. 184, No. 5
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.5.1395–1401.2002

Effects of a Squalene Epoxidase Inhibitor, Terbinafine, on Ether Lipid Biosyntheses in a Thermoacidophilic Archaeon, *Thermoplasma acidophilum*

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Tetraether polar lipid biosynthesis

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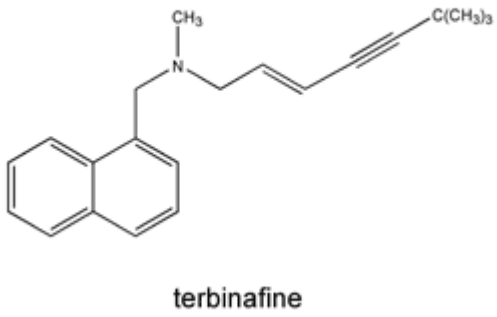
DGGGP
digeranylgeranylglyceryl phosphate

^{32}P orthophosphate pulse labeling: faster incorporation into diether polar lipids than into tetraether

Radioactivity in diether polar lipids declined; radioactivity increased in tetraether polar lipids

3 reactions required:
- **head to head condensation**
- **saturation of hydrophobic chains**
- **modification of polar head group**

Terbinafine



terbinafine

-Synthetic antifungal allylamine

-Inhibits squalene epoxidase in eukaryotes, disrupting early steps in steroid biosynthesis

-Mechanism unclear; may interfere with a lipid-binding domain of squalene epoxidase

Terbinafine:

Little effect on growth rate,
saturation cell density of
E.coli (A) or *H. halobium* (B)

O = 0μg/ml terbinafine
■ = 100 μg/ml

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T. acidophilum: reversible inhibition

90-95% membrane tetraether lipids

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FIG. 3. (A) Effect of terbinafine on the growth of *T. acidophilum*. Concentration of inhibitors: 0 (open circles), 1 (solid circles), 10 (open squares), and 100 μg/ml (solid squares). (B) Effect of terbinafine after washing the cells with fresh medium. *T. acidophilum* cells were incubated in the presence of 0 (circles), 100 (open and solid triangles), or 600 (open and solid squares) μg of terbinafine/ml at 57°C for 1 h. Then, the cells were washed with fresh medium and further cultivated for the times indicated in the absence (open symbols) or presence (solid triangles) of the compound.

Pulse and chase

Pulse: ^{14}C labeled mevalonic acid

Chase: Cells washed, incubated with or without terbinafine

Lipids extracted, analyzed by thin-layer chromatography

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1. H. halobium lipids

2.T. acidophilum lipids

3-5. Pulse of labeled mevalonic acid, *T. acidophilum*

6-12. Terbinafine added during pulse-labeling or chase

(6,7,11,12: terbinafine present)

Results

Terbinafine inhibits tetraether lipid biosynthesis in *T. acidophilum*

Inhibition is concentration dependent and reversible

Conclusions

Tetraether lipids are likely synthesized from the lipid that accumulated in the presence of terbinafine, the “PTL”

Terbinafine inhibits synthesis of tetraether lipids from PTL; inhibition is concentration-dependent and reversible

PTL = DGGGP?

differences in head group
acid labile vs. stable

Similarity between squalene epoxidase and an enzyme that catalyzes condensation in Archaea?

Evolutionary implications?

Importance of the isopropylidene terminal of geranylgeranyl group for the formation of tetraether lipid in methanogenic archaea

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How are the critical C-C bonds formed in macrocyclic lipids of Archaea?

Speculated mechanism (initial):

> “Intermolecular acid catalyzed condensation at the saturation stage after isomerization of double bond”

Feeding experiments

Deuterium labeled digeranygeranylglycerol analogs synthesized

Methanothermobacter thermautotrophicus

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diphytanylglycerol

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Feeding experiment

- Deuterated substrates added to culture
- 80% H_2 , 20% CO_2 gas mixture
- 65°C, 5 days

Harvested, lipids purified, solvolysis of polar head groups

Repetitive chromatography
Core lipids converted to benzoates
Benzoates analyzed by ^2H NMR

Results

No uptake of 6

4 incorporated into both dibyiphytanylglycerol (1)& the 72 membered lipid (3)

5 incorporated into (1) but not (3)

Interpretations

Double bond migration is not a trigger for C-C bond formation in biosynthesis of macrocyclic lipids

Presence of Δ14 double bond of digeranyl groups is crucial to forming macrocyclic lipids

hydrogenation of double bond at the far end may be a branching point leading to diphytanyl-glycerol lipid or 72-membered lipid

Concerns?

mechanism

Genome Research

Biosynthesis of Isoprenoids via Mevalonate in Archaea: The Lost Pathway

Arian Smit and Arcady Mushegian

Genome Res. 2000 10: 1468-1484

Figure 1 removed due to copyright considerations.

- **Figure 1** The **mevalonate** and deoxy-D-xylulose (DXP) pathways of isoprenoid biosynthesis. Gene names and GenBank accession nos. for the prototype yeast proteins of the **mevalonate** pathway are shown. Green shading indicates genes orthologous to the yeast prototypes. Gene displacements are shown in yellow or, when the replacing enzymes have not been characterized, in red. Blue shading indicates the enzymes of the DXP pathway. No shading indicates that these functions are more likely to be absent in a given genus. Compounds are indicated by Roman numerals: I, acetyl-CoA; II, acetoacetyl-CoA; III, hydroxy-3-methylglutaryl-CoA; IV, **mevalonate**; V, phospho**mevalonate**; VI, diphospho**mevalonate**; VII, isopentenyl pyrophosphate; VIII, dimethylallyl pyrophosphate; IX, geranylpyrophosphate; X, pyruvate; XI, glyceraldehyde 3-phosphate; XII, 2-deoxy-D-xylulose 5-phosphate; XIII, 2*C*-methyl-D-erythritol 4-phosphate; XIV, 4-diphosphocytidyl-2*C*-methyl-D-erythritol; XV, 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate; XVI, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate; XVII, isopentenyl monophosphate. Arabic numerals indicate enzymes. **Mevalonate** pathway: 1, acetoacyl-CoA synthetase; 2, hydroxy-3-methylglutaryl-CoA synthase; 3, hydroxy-3-methylglutaryl-CoA reductase; 4, **mevalonate** kinase; 5, phospho**mevalonate** kinase; 6, diphospho**mevalonate** decarboxylase; 7, isopentenyl pyrophosphate delta-isomerase; 8, geranyl pyrophosphate synthase family (the sign indicates that orthologs and paralogs are not well distinguished in this family, which is compatible with the observation that substrate specificity of these enzymes is modulated easily by small number of point mutations). DXP pathway: 9, deoxy-D-xylulose phosphate synthase; 10, deoxy-D-xylulose phosphate reductoisomerase; 11, 2*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase (YgbP); 12, isopentenyl monophosphate kinase; 13, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase (YgbB).

Figure 2 removed due to copyright considerations.

Figure 2 Conserved sequence motifs in mevalonate pathway enzymes. Blocks of high-sequence similarity are shown. Unique identifiers in SWISSPROT or GenBank are given for each sequence. Yellow shading indicates conserved bulky hydrophobic residues (I, L, F, M, V, Y, and W), red type indicates conserved small side chain residues (A, G, and S), and blue type indicates other conserved residues. Secondary structures predicted with reliability of eight or higher (PHD program) are shown; h indicates a helix, and s indicates a strand. (*A*) Diphosphomevalonate kinase belongs to the galactokinase superfamily. Secondary structures for yeast phosphomevalonate kinase (ERG8) and diphosphomevalonate decarboxylase (ERG19) predicted with reliability of eight or higher are shown. (*B*) Conserved ATP-binding motifs of nucleotide monophosphate kinase type in metazoan phosphomevalonate kinases. Secondary structure elements observed in the T4 bacteriophage deoxynucleoside monophosphate kinase (pdb code 1DEL) and predicted for human phosphomevalonate kinase (PMKA_HUMAN) are shown. Green shading indicates residues located within 3å distance from the bound ADP. (*C*) MutT-like pyrophosphate-binding motifs in isopentenyl pyrophosphate delta-isomerases. Secondary structure elements observed in *Escherichia coli* MutT protein (pdb code 1TUM) and predicted for yeast IPPI (IDI1_YEAST) are shown.

Figure 3 removed due to copyright considerations.

Figure 3 (*A*) Phylogenetic tree of the galactokinase superfamily. The results of neighbor joining analysis are shown, which fully correspond to the maximum likelihood data. The tree was built with 116 galactokinase superfamily members, all < 90% identical to one another. Forty-seven less-informative proteins have been weeded out afterwards. Thick lines indicate a bootstrap value >75% for the corresponding node. Eukaryotic branches are in blue, eubacterial in green, and archaeal in red lines. Three orphan archaeal-specific families within the superfamily stand out, and may include phosphomevalonate kinases, isopentenyl monophosphate kinases, and, less likely, diphosphomevalonate decarboxylases (see text).

Figure 4 removed due to copyright considerations.

Figure 4 Conserved strings include genes of the mevalonate pathway in archaea and bacteria. Blocks connected by an arrow indicate neighboring genes with a common transcriptional orientation, possibly representing operons. Blocks containing numbers represent known mevalonate pathway genes, as in Figure 1. Blocks designated as 2 indicate hydroxy-3-methylglutaryl-CoA synthase; 3 indicates 3-hydroxy-3-methylglutaryl-CoA reductase; 4 indicates mevalonate kinase; 5 indicates yeast-like phosphomevalonate kinase; 6 indicates diphosphomevalonate decarboxylase; and 8 indicates octaprenyl-diphosphate synthase, a member of the geranyl pyrophosphate synthase family. Other designations: A indicates ancient conserved protein (COG #1355), K indicates putative kinase related to uridylate- and acetylglutamate kinases, Cindicatescarotenoid biosynthesis protein (flavin-dependent oxidoreductase), and H indicates putative metal-dependent hydrolase. *Pyrococcus abyssi* has the same structure as *Pyrococcus horikoshii*, with one gene insertion between ancient conserved protein and mevalonate kinase. In *Streptococcus pyogenes*, genes 2 and 3 are flanking the mevalonate kinase operon, but are transcribed in opposite orientation. The mevalonate kinase gene has not been sequenced yet in *Sulfolobus solfataricus*. The putative metal-dependent hydrolase has no orthologs in *Aeropyrum* and *Archaeoglobus*. GenBank identification nos. are given below the boxes, where available. Apparently-missing GI numbers in strings correspond to overlapping genes, typically short open reading frames (ORFs) on the opposite strand.

Figure 5 removed due to copyright considerations.

Figure 5 Frequent horizontal transfers in the evolution of isoprenoid biosynthesis. Alternative pathways and displacements are color coded, mostly according to Fig. 1. Evolution of fatty acid biosynthesis has not been dissected in detail but is also shown for comparative purposes. Green lines, mevalonate pathway; blue lines, DXPS pathway; yellow, displacement of phosphomevalonate kinase in metazoans; red arrow and red-and-green line, displacement of three enzymes of mevalonate pathway, resulting in the chimeric pathway in archaea. Gray arrows, transfer of fatty acid biosynthesis genes to eukaryotes (solid line) and to selected archaea (double line). Green double line represents the transfer of HMG-CoA reductase to *Vibrio cholerae*, apparently from archaea (Heidelberg et al. 2000). The donor of the mevalonate pathway to *Borrelia* and cocci is unknown; a yet undiscovered isopentenyl diphosphate isomerase enzyme in these species may be shared with other bacteria (blue checkers) or with archaea (red checkers).