Methods for Analyzing Archaeal Lipids

Augusta Dibbell
12.458
11-10-2006

Detection of Regioisomeric Macrocyclic Tetraethers in the Lipids of *Methanobacterium thermoautotrophicum* and other Archaeal Organisms

Otto Gräther and Duilio Arigoni. *J. Chem. Soc., Chem. Commun*. 405-406 (1995)

Chemical degradation of GDGTs from cultured microorganisms revealed a near-racemic mixture of caldarchaeol to isocaldarchaeol (45:55).

Special methods for the analysis of ether lipid structure and metabolism in archaea.

Yosuke Koga & Hiroyuki Morii. *Analytical Biochemistry* 348, (2006): 1-14.

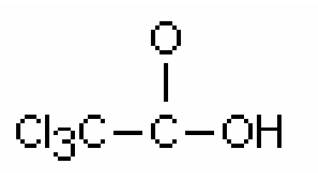
An overview of chemical methods used to analyze archaeal lipids, focusing on diether lipids.

Extraction using an acidified Bligh-Dyer method.

Extraction and Composition of Polar Lipids from the Archaebacterium, *Methanobacterium thermoautotrophicum*: Effective Extraction of Tetraether Lipids by an Acidified Solvent

Nishihara, Masateru, Yosuke Koga., and *J. Biochem*. **101**, (1987): 997-1005.

Instead of using a regular Bligh-Dyer solvent mixture for the extraction ($MeOH/CHCl_3/H_2O$, 2:1:0.8), the water is replaced with a 5% TCA (trichloroacetic acid) aqueous solution.



TCA is a strong protein denaturing agent and commonly used to precipitate DNA and RNA in biochemistry.

Lipids comprised 0.98% of the cell dry weight with the regular solvent mixture and 5.58% with the TCA mixture.

Nishihara and Koga identified polar archaeal lipids using TLC (Thin Layer Chromatography).

Solvent A: chloroform, methanol, 7M aqueous ammonia (60:35:8)

Solvent B: chloroform, methanol, acetic acid, water (85:30:15:5)

Spray reagents used to identify IPLs (Intact Polar Membrane Lipids):

Ninhydrin	amino groups
Acid molybdate	phospholipids
0.5% α-naphthol reagent	glycolipids
Periodate-Schiff reagent	vicinal hydroxyl groups
30% H_2SO_4	all lipids

Solvent A

Image removed due to copyright considerations.

Solvent B

acid molybdate positive
α– naphthol positive
ninhydrin positive

Fig. 2. TLC chromatogram of total lipid of *M. thermoautophicum*. Details of the development of lipids and the detection of each lipid are given in " MATERIALS AND METHODS."

Wet chemistry techniques for the preparation of core lipids.

HCL Methanolysis:

5% HCl in MeOH at 100°C for 3 h

Cleaves glycosidic bonds, but will not cleave phosphodiester bonds in diether lipids.

Acetolysis with acid methanolysis:

Acetic acid, acetic anhydride (3:2) at 160°C for 16 h

Cleaves phosphodiester bonds, forming an acetylated core lipid which is removed by subsequent methanolysis.

HF cleavage with mild HCl methanolysis:

46% HF at 0°C for 24 h then 0.18% HCl at 50°C for 24 h

Cleaves phosphodiester bonds while preserving hydroxyarachaeols.

Ether cleavage methods.

HI cleavage:

47% HI under reflux for 24 h

Preferred by Koga and Morii because it reportedly gives a sharper peak in GC analysis.

BBr3/BCl3 + Lithium aluminium hydride (LAH):

Cleaves ether bonds while preserving hydroxyl groups.

Kates, et al. *Biochim. Biophys. Acta,* **98** (1965): 252-268.

LAH cleavage:

100°C for 90 min in the presence of LAH

Cleaves allyl ether bonds, yielding unsaturated

Acid methanolysis:

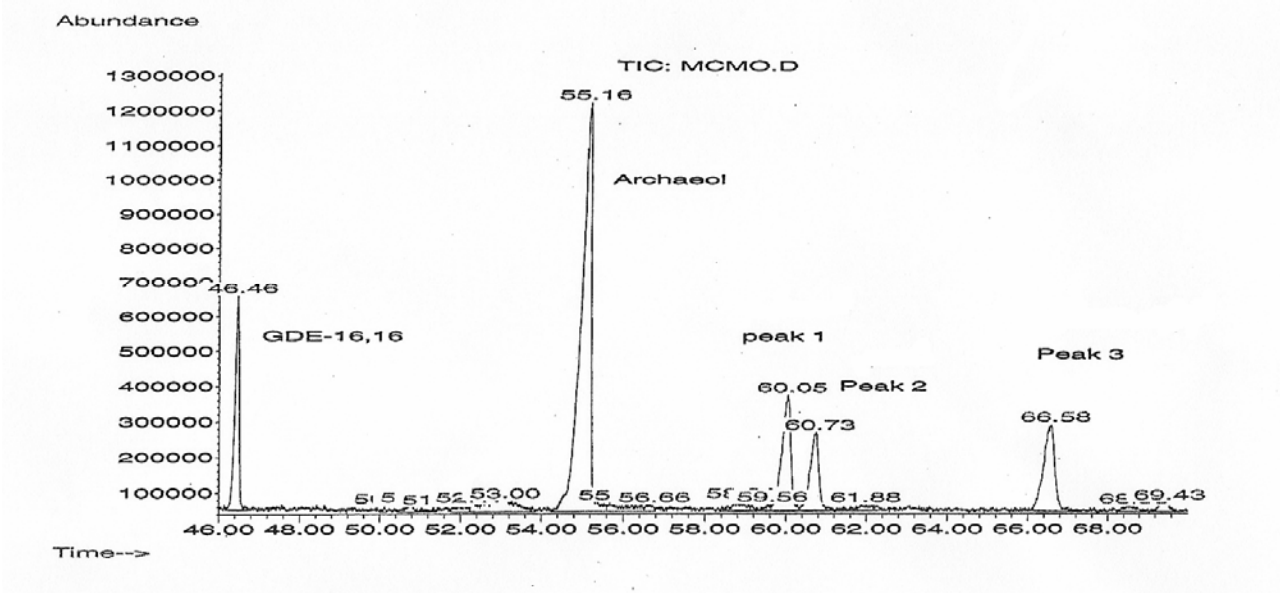
5% HCl in methanol 80°C for 1 h

Allyl ether bonds cleaved, while non-allyl ether bonds remain intact.

Gas Chromatography Mass Spectroscopy of Archaeal Lipids

Archaeal diether lipids are GC-amenable after derivatization. Archaeal tetraether lipids are not GC-amenable, and ether cleavage is required to resolve the alkyl chains bound between the glycerol moieties.

Chromatogram of Methanococcus TMS ether lipids. Sample was recovered from TLC plate origin zone after mild acid methanolysis of TLE. GC conditions. 60oC to 200o @ 10o/min; 200 to 300 @ 4o/min; hold 60 min. Column DB5ms.



sn-2-hydroxyarchaeol

Image removed due to copyright considerations.

Hinrichs, et al. *Organic Geochemistry* **31,** (2000): 1685-1701

Ether Cleavage Products

Image removed due to copyright considerations.

Delong, et al. *Applied and Environmental Microbiology* **64,** no. 3 (1998): 1133-1138.

HPLC (High Performance Liquid Chromatography) Overview

Types of Columns:

Normal Phase:

Uses a polar stationary phase and a non-polar mobile phase. Hydrophobic compounds elute first.

Reverse Phase:

Uses a non-polar stationary phase and a polar mobile phase. Hydrophilic compounds elute first

Size Exclusion:

Uses a stationary phase composed of porous beads.

Ion Exchange:

Operates on the basis of selective exchange of ions in the sample with counterions in the stationary phase.

Liquid-Liquid:

Components in the sample capable of hydrogen bonding with the stationary phase are retained longer.

Ionization Sources:

Electrospray Ionization (ESI):

Image removed due to copyright considerations.

Atmospheric Pressure Chemical Ionization (APCI):

The mobile phase is heated to above 400°C and the aerosol cloud is subjected to a corona discharge which creates ions.

Image removed due to copyright considerations.

Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry.

Hopmans, et al. *Rapid Commun. Mass Spectrom* **14,** (2000): 585-589.

Uses a normal phase column to separate GDGTs of different unsaturations, allowing for rapid determination of the relative distribution of intact GDGTs.

Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology.

Sturt, et al. *Rapid Commun. Mass Spectrom* **18,** (2004): 617-628.

Uses a normal phase column to separate and identify IPLs of prokaryotic communities. A combination of positive and negative ion modes can distinguish between diacyl, diether, and acyl-ether glycerol backbones as well as identify the polar head groups of IPLs.

Review and re-analysis of domain-specific 16S primers

Baker, et al. *Journal of Microbiological Methods* **55,** (2003): 541-555.

Image removed due to copyright considerations.

PCR Overview:

1) DNA denaturing: DNA and primers heated to 94-98°C for 1-5 min.

2) Annealing: Temperature lowers so primers can attach themselves to single strands of DNA. Usually 5°C below the melting temp. of the primers for 1-2 min.

3) Polymerization: DNA polymerase is added, binds to the primer, and replicates the DNA. *Taq polymerase* replicates optimally at 72°C. This step typically lasts 10-15 min.

Baker et al. note that current “universal” primers for prokaryotes do not amplify Archaea well, introducing bias when constructing libraries of environmental samples.

Using as many available genomes as possible, they propose new primers designed to amplify prokaryotes from the Archaeal domain: A571F/UA1204R and A751F/UA1406R.

These new primers were tested, and observed to amplify more sequences from environmental samples than previously reported, but did not amplify sequences from *E. coli*.

Inosine residues and degenerate bases were used in primer design when mismatches occurred in documented genomes in order to maximize complementarity.

^{1}H-NMR Review

Proton Chemical Shift Ranges

Image removed due to copyright considerations.