COMMUNICATION

Variation in Molecular Species of Polar Lipids from *Thermoplasma acidophilum* Depends on Growth Temperature

Ikuko Uda[a,*], Akihiko Sugai[a], Yuko H. Itoh[b], and Toshihiro Itoh[a]

[a]Division of Chemistry, Center for Natural Science, Kitasato University, Kanagawa 228-8555, Japan, and
[b]Department of Bioengineering, Faculty of Engineering, Soka University, Tokyo 192-8577, Japan

ABSTRACT: Five types of molecular species of C_{40} isoprenoid chains, having different numbers of cyclopentane rings, were detected in the ether core lipid of *Thermoplasma acidophilum*. The average cyclization number of the hydrocarbon chains in the lipids increased with increasing growth temperatures.

Paper no. L8633 in *Lipids 36*, 103–105 (January 2001).

The membrane lipid structures of Archaea are composed of different lengths of isoprenoid chains with an ether linkage to glycerol in the *sn*-2,3 configuration (1,2). The hydrocarbon chain constituents of ether lipids are commonly the C_{20}, C_{25}, and C_{40} isoprenoid chains. In the thermoacidophilic Archaea, caldarchaeol (2,2′,3,3′-tetra-*O*-dibiphytanyl-*sn*-diglycerol), which contains 2 mol of C_{40} isoprenoid, is the major core lipid (3,4). Five types of molecular species, classified by the number of cyclopentane rings, have been detected in the core lipid of *Thermoplasma acidophilum* and other extreme thermophiles including *Sulfolobus solfataricus* (5–7); these are the acyclic-, monocyclic-, bicyclic-, tricyclic-, and tetracyclic-C_{40} hydrocarbons. De Rosa *et al.* (8) reported that cyclization of the chain increased systematically with the growth temperature from 75 to 89°C in *S. solfataricus,* which is an extreme thermoacidophilic Crenarchaeon belonging to the order Sulfolobales. *Sulfolobus solfataricus* can grow from 50 to 87°C and optimally at 87°C. *Thermoplasma acidophilum* belongs to the Euryarchaeota, a subdomain of Archaea, and grows from 45 to 62°C (optimally at 59°C) (9). The cell is wall-less, and the major core lipid of the cell membrane is caldarchaeol (10).

In contrast to the report on *S. solfataricus* (8), Yang and Haug *et al.* (11) reported that the average cyclization number of the total lipids of *T. acidophilum* grown at 37°C was greater than that grown at 56°C. Recently, at least five types of neutral lipids and eight types of acidic lipids have been identified in *T. acidophilum,* and most of their structures have been characterized (12–14).

This report describes the effect of growth temperature on the degree of cyclization of the major lipids, five neutral lipids, and four acidic lipids in *T. acidophilum*, in all of which the core lipid is caldarchaeol.

EXPERIMENTAL PROCEDURES

Thermoplasma acidophilum (ATCC 27658) was grown aerobically with low-speed stirring at 40, 50, and 60°C (±1°C) at pH 2.0. The medium (1.5 L, wt/vol) consisted of inorganic salts, 0.1% yeast extract, and 1% glucose in 2-L flasks, as previously described (13). Cells grown at 60°C were used as inoculum. Lipid extraction from the cells and the preparation of the neutral lipids and acidic lipids from the total lipids were carried out as previously described (13). As complete lipid extraction is necessary for reproducible results, the whole cells were treated with acidic methanolysis during the first step. Five neutral glycolipids (GL-1a, GL-1b, GL-2a, GL-2b, and GL-2c), and four acidic phosphoglycolipids (GPL-A, GPL-B, GPL-C, and GPL-D) were fractionated by the combination of column chromatography and preparative thin-layer chromatography (TLC). All of the core lipids from these intact lipids were co-chromatographed with caldarchaeol (R_f = 0.55) by TLC analysis. Each lipid was purified by preparative TLC using boronic acid-impregnated high-performance TLC (HPTLC) plates of silica gel (Merck, Darmstdt, Germany). The HPTLC plates were developed with either chloroform/methanol/water (75:25:2, by vol) for purification of each neutral lipid, chloroform/methanol/0.2% $CaCl_2$ (55:45:7, by vol) or chloroform/methanol/1 M aqueous ammonia (65:35:5, by vol) for each acidic lipid.

The core lipids were obtained from whole cells or intact lipids by acid methanolysis. The core lipids bound to the intact polar lipids were completely cleaved and extracted by a chloroform/methanol mixture from the methanolyzate. TLC analysis of the core lipids was performed using an HPTLC plate of silica gel and developed with hexane/diethyl ether/acetic acid (60:40:2, by vol). Core lipids were detected by spraying with 18 M H_2SO_4 followed by heating at 150°C. Preparation of the hydrocarbon chains from the core lipids were obtained by HI degradation as described previously (13). The hydrocarbon chains were analyzed by gas–liquid chromatography (GLC) and gas chromatography–mass spectrometry (GC–MS). GLC was performed on a Hitachi 163 gas chromatograph (Hitachi, Tokyo, Japan) equipped with a flame-ionization detection system. Hydrocarbon chains were analyzed on 3% Dexsil 300 in a glass column (3 mm × 1 m)

*To whom correspondence should be addressed at Division of Chemistry, Center for Natural Science, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan. E-mail: uda@clas.kitasato-u.ac.jp

Abbreviations: EI, electron ionization; GC–MS, gas chromatography–mass spectrometry; GLC, gas–liquid chromatography; HPTLC, high-performance thin-layer chromatography; TLC, thin-layer chromatography.

TABLE 1
Distribution of Cyclopentane Rings in Core Lipids of Whole Cell, Neutral Lipids, and Acidic Lipids from *Thermoplasma acidophilum* Grown at 40–60°C

Temperature of growth	% of C_{40} hydrocarbon					Average degree
(°C)	Acyclic	Monocyclic	Bicyclic	Tricyclic	Tetracyclic	of cyclization[a]
Whole cell						
40	4.9	32.7	62.4	ND[b]	ND	1.6 ± 0.06
50	2.3	20.7	71.9	5.2	ND	1.8 ± 0.09
60	0.7	7.0	77.6	14.6	<0.1	2.1 ± 0.04
Neutral lipids						
40	16.9	36.1	46.6	0.4	ND	1.3 ± 0.12
50	7.8	23.0	66.3	2.9	ND	1.6 ± 0.12
60	2.9	5.1	81.8	10.2	ND	2.0 ± 0.08
Acidic lipids						
40	6.0	31.2	55.3	7.6	ND	1.6 ± 0.02
50	1.7	14.8	76.1	7.4	ND	1.9 ± 0.07
60	0.3	4.4	76.9	17.8	0.6	2.1 ± 0.02

[a]Average degree of cyclization: (%monocyclic + 2 × %bicyclic + 3 × %tricyclic + 4 × %tetracyclic) × 10^{-2}.
[b]ND, not detected (<0.01).

from 100 to 300°C at the rate of 15°C/min. GC–MS electron ionization (EI) was carried out using a gas chromatograph–mass spectrometer (JMS-AX505H; JEOL, Tokyo, Japan) with a capillary column (DB-1, 0.53 mm × 15 m). The accelerating voltage was 3.0 kV, and the primary beam for the bombardment was 6.0 keV of xenon.

TABLE 2
Distribution of Cyclopentane Rings in Glycolipids (GL) and Glycophospholipids (GPL) from *T. acidophilum*

Temperature of growth	% of C_{40} hydrocarbon					Average degree
(°C)	Acyclic	Monocyclic	Bicyclic	Tricyclic	Tetracyclic	of cyclization[a]
GL-1a						
40	7.6	47.2	44.1	1.1	ND	1.4
50	3.5	29.7	64.8	2.0	ND	1.7
60	0.4	5.2	80.8	13.4	0.2	2.1
GL-1b						
40	14.8	53.0	32.2	ND	ND	1.2
50	7.8	23.0	66.3	2.9	ND	1.6
60	9.3	21.3	69.4	ND	ND	1.6
GL-2a						
40	1.3	29.7	67.3	1.7	ND	1.7
50	0.6	15.8	82.9	0.7	ND	1.8
60	0.4	2.7	91.4	5.5	ND	2.0
GL-2b						
40	1.8	20.4	77.5	0.3	ND	1.8
50	1.8	8.5	85.1	4.6	ND	1.9
60	0.8	1.7	87.5	10.1	ND	2.1
GL-2c						
40	44.2	19.6	36.3	ND	ND	0.9
50	36.8	13.0	47.1	3.2	ND	1.2
60	21.7	7.2	56.3	14.8	ND	1.6
GPL-A						
40	3.4	44.6	52.1	ND	ND	1.5
50	1.5	8.8	85.0	4.7	ND	1.9
60	Trace	2.6	83.5	13.3	0.7	2.1
GPL-B						
40	4.6	22.6	72.6	0.3	ND	1.7
50	2.0	8.1	86.6	3.3	ND	1.9
60	Trace	2.6	88.6	8.2	0.6	2.1
GPL-C						
40	5.6	35.8	58.7	ND	ND	1.5
50	Trace	14.0	82.1	3.9	ND	1.9
60	0.8	5.1	78.7	14.7	0.7	2.1
GPL-D						
40	Trace	15.6	80.8	3.6	ND	1.9
50	Trace	11.4	81.9	6.8	ND	2.0
60	Trace	3.4	83.0	13.6	ND	2.1

[a]Average degree of cyclization: (%monocyclic + 2 × %bicyclic + 3 × %tricyclic + 4 × %tetracyclic) × 10^{-2}. For abbreviations see Table 1.

RESULTS AND DISCUSSION

Freeze-dried whole cells were directly methanolyzed, then prepared as hydrocarbon chains. The gas chromatogram of the hydrocarbon chain from the core lipid (caldarchaeol) showed five peaks corresponding to the C_{40} hydrocarbon chains detected in *S. solfataricus* (8). The peaks for $[M]^+$ obtained from the GC–MS EI spectra at *m/z* 562, 560, 558, 556, and 554 were identified as $C_{40}H_{82}$ (acyclic), $C_{40}H_{80}$ (monocyclic), $C_{40}H_{78}$ (bicyclic), $C_{40}H_{76}$ (tricyclic), and $C_{40}H_{74}$ (tetracyclic), respectively. The distribution of molecular species of the C_{40} isoprenoid and the average cyclization in the whole cells and fractionated lipids of *T. acidophilum* grown at 40, 50, and 60°C, are shown in Table 1. From these results, the average cyclization of the C_{40} hydrocarbon chains increased with the increasing growth temperature. These results are similar to those from *S. solfataricus*, but opposite to that of a previous report on *T. acidophilum* (11).

Distribution of the molecular species of the C_{40} isoprenoid and average cyclization from the neutral glycolipids and acidic phosphoglycolipids are shown in Table 2. An increasing degree of cyclization was seen in all the lipids examined. The existence of a lipopolysaccharide with 24 mannose residues in its polar head portion has been reported in *T. acidophilum* (15,16). In our experiments, the lipopolysaccharide was not detected on the TLC plate, probably because of its behavior based on high molecular weight and high polarity. The efficiency of extraction for the high molecular weight lipids depends on the extraction solvents, which should be affected by the cyclization number in the total lipids. The previous contradictory result (11) from *T. acidophilum* might have been related to the extraction conditions. The phosphoglycolipids are more cyclized than the glycolipids. The reason for this might be that the physical volume of the polar head groups of the phosphoglycolipids are larger than that of the glycolipids; the volume of the core lipid as a membrane anchor part needs to become larger for controlling the distance between the polar heads.

Changing the number of cyclopentane rings in the core lipids of thermoacidophilic Archaea could maintain stable fluidity of the membrane against environmental temperature changes, similar to the change in the unsaturation of the fatty acylester lipids of Eukarya and Bacteria (17,18).

The main molecular species of the C_{40} hydrocarbon chain of the major cellular lipid GPL-A and total lipids from *T. acidophilum* grown at its optimum of 60°C in 5 L of medium without stirring contains one cyclopentane ring (C_{40} monocyclic). In the case of the cultivation carried out in a 1.5-L medium with stirring at 60°C, the main molecular species of the hydrocarbon chain was the bicyclic C_{40} hydrocarbon. Thus cyclization might be a response not only to temperature but also to physical stimulation. *Thermoplasma acidophilum* is wall-less and its cell membrane directly faces into the environment. The stability of the membrane structure also might be controlled by cyclization of hydrocarbon chains against the physical stress, like stirring cultivation.

REFERENCES

1. Gambacorta, A., Gliozzi, A., and De Rosa, M. (1995) Archaeal Lipids and Their Biotechnological Applications, *World J. Microbiol. Biotechnol. 11*, 115–131.
2. Sprott, G.D. (1992) Structures of Archaebacterial Membrane Lipids, *J. Bioenerg. Biomembr. 24*, 555–566.
3. Nishihara, M., Morii, H., and Koga, Y. (1987) Structure Determination of a Quartet of Novel Tetraether Lipids from *Methanobacterium thermoautotrophicum*, *J. Biochem. 101*, 1007–1015.
4. De Rosa, M., and Gambacorta, A. (1988) The Lipids of Archaebacteria, *Prog. Lipid Res. 27*, 153–175.
5. Langworthy, T.A. (1977) Long-chain Diglycerol Tetraether from *Thermoplasma acidophilum*, *Biochim. Biophys. Acta 487*, 37–50.
6. De Rosa, M., De Rosa, S., and Gambacorta, A. (1977) ^{13}C-NMR Assignments and Biosynthetic Data for the Ether Lipids of *Caldariella, Phytochemistry 16*, 1909–1912.
7. De Rosa, M., Gambacorta, A., Nicolaus, B., Sodano S., and Bu'Lock, J.D. (1980) Structural Regularities in Tetraether Lipids of *Caldariella* and Their Biosynthetic and Phyletic Implications, *Phytochemistry 19*, 833–836.
8. De Rosa, M., Esposito, E., Gambacorta, A., Nicolaus, B., and Bu'Lock, J.D. (1980) Effects of Temperature on Their Lipid Composition of *Caldariella acidophila*, *Phytochemistry 19*, 827–831.
9. Darland, G., Brock, T.D., Samsonoff, W., and Conti, S.F. (1970) A Thermophilic, Acidophilic Mycoplasma Isolated from a Coal Refuse Pile, *Science 170*, 1416–1418.
10. Langworthy, T.A., Smith, P.F., and Mayberry, W.R. (1972) Lipids of *Thermoplasma acidophilum*, *J. Bacteriol. 112*, 1193–1200.
11. Yang, L.L., and Haug, A. (1979) Structure of Membrane Lipids and Physico-biochemical Properties of the Plasma Membrane from *Thermoplasma acidophilum*, Adapted to Growth at 37°C, *Biochim. Biophys. Acta 573*, 308–320.
12. Swain, M., Brisson, J.-R., Sprott, G.D., Cooper, F.P., and Patel, G.B. (1997) Identification of β-L-Gulose as the Sugar Moiety of the Main Polar Lipid *Thermoplasma acidophilum, Biochim. Biophys. Acta 1345*, 56–64.
13. Uda, I., Sugai, A., Kon, K., Ando, S., Itoh, Y.H., and Itoh, T. (1999) Isolation and Characterization of Novel Neutral Glycolipids from *Thermoplasma acidophilum, Biochim. Biophys. Acta 1439*, 363–370.
14. Uda, I., Sugai, A., Shimizu, A., Itoh, Y.H., and Itoh, T. (2000) Glucosylcaldarchaetidylglycerol, a Minor Phosphoglycolipid from *Thermoplasma acidophilum*, *Biochim. Biophys. Acta 1484*, 83–86.
15. Mayberry-Carson, K.J., Langworthy, T.A., Mayberry, W.R., and Smith, P.F. (1974) A New Class of Lipopolysaccharide from *Thermoplasma acidophilum, Biochim. Biophys. Acta 360*, 217–229.
16. Smith, P.F. (1980) Sequence and Glucosidic Bond Arrangement of Sugars in Lipopolysaccharide from *Thermoplasma acidophilum, Biochim. Biophys. Acta 619*, 367–373.
17. Nozawa, Y., Iida, H., Fukushima, H., Ohki, K., and Ohnishi, S. (1974) Studies on *Tetrahymena* Membranes: Temperature-Induced Alterations in Fatty Acid Composition of Various Membrane Fractions in *Tetrahymena pyriformis* and Its Effect on Membrane Fluidity as Inferred by Spin-Label Study, *Biochim. Biophys. Acta 367*, 134–147.
18. Kito, M., Aibara, S., Kato, M., Ishinaga, M., and Hata, T. (1973) Effect of Changes in Fatty Acid Composition of Phospholipid Species on the β-Galactoside Transport System of *Escherichia coli* K-12, *Biochim. Biophys. Acta 298*, 69–74.

[Received September 22, 2000, and in revised form November 10, 2000; revision accepted November 15, 2000]