APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2006, p. 4419–4422 Vol. 72, No. 6
0099-2240/06/$08.00+0 doi:10.1128/AEM.00191-06

Thermophilic Temperature Optimum for Crenarchaeol Synthesis and Its Implication for Archaeal Evolution

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Received 24 January 2006/Accepted 20 March 2006

The isoprenoid lipid crenarchaeol is widespread in hot springs of California and Nevada. Terrestrial and marine data together suggest a maximum relative abundance of crenarchaeol at ~40°C. This warm temperature optimum may have facilitated colonization of the ocean by (hyper)thermophilic *Archaea* and the major marine radiation of *Crenarchaeota*.

Crenarchaeota synthesize membranes in which the major lipids are glycerol dialkyl glycerol tetraethers (GDGTs) (5, 14, 15). In thermophilic archaea (8, 26) and in the ocean (22, 23) the number of cyclopentane rings increases with increasing surface water temperature (20, 22, 23). Crenarchaeol is an unusual GDGT first identified in marine sediments (1, 2, 4, 10, 18, 21, 24, 29, 30). It contains four cyclopentyl rings and, uniquely among GDGTs, it also contains one cyclohexyl ring (1). Results from molecular models show that the cyclohexyl moiety adds molecular volume, thereby enhancing flexibility at lower temperatures (1). However, contrary to physical expectations, we observed that crenarchaeol is not a more abundant fraction of total GDGTs in samples from progressively colder environments (references 1 and 24 and this study).

Recently, crenarchaeol was found in high relative abundance in terrestrial hot springs (19). Therefore, we sought to explore the distribution of crenarchaeol in environmental samples across a broad range of temperatures. The marine and terrestrial data for the relative abundance of crenarchaeol now span psychrophilic to hyperthermophilic communities ranging from <10°C to 87°C, indicating a broad biosynthetic distribution of the compound. The data suggest an evolutionary history of crenarchaeol that may be longer and more complex than its distribution in the modern ocean indicates.

Lipid extraction and liquid chromatography-mass spectrometry. Total lipids were extracted from mat material collected from hot springs by using established procedures (19, 30). The lipids were acid hydrolyzed and screened by high-performance liquid chromatography–mass spectrometry. The column was Zorbax NH_2 (custom 2.1 mm by 150 mm; 5-μm particle size; isocratic 1.3% isopropanol in hexane; 30°C). Conditions for atmospheric-pressure chemical ionization–mass spectromety were as stated previously (11, 19).

Distribution of crenarchaeol in California and Nevada hot springs. Seventeen samples were analyzed from 11 hot-spring locations in California and Nevada and from one spring in Thailand (Table 1). Additional data for marine samples from Santa Monica Basin, Santa Barbara Basin, and Cariaco Basin and from the literature are shown in Table 2.

For the hot-spring samples, correlations were explored between the relative abundances of all GDGTs and each of the environmental parameters, including temperature, pH, and alkalinity. Crenarchaeol (I) (Fig. 1) was the only GDGT that showed a negative slope with temperature ($R^2 = 0.64$) (Fig. 2a). GDGT II exhibited no relationship to temperature ($R^2 = 0.03$), and the correlation lines for all other numbers of cyclopentyl rings (III to VII) had positive slopes ($R^2 = 0.45$ to 0.57) (Fig. 2a).

Our discovery of crenarchaeol in a wide range of terrestrial hot springs has significant ecological and evolutionary implications. Crenarchaeol is abundant in marine environments and accounts for up to 46% of total GDGTs in marine sediments having temperatures below 20°C (24) and up to 60% of total GDGTs in surface waters at 26°C (12). There is also approximately 60% crenarchaeol in the GDGTs of the symbiont *Cenarchaeum symbiosium* (1, 21).

In hot springs, two of our samples contained 100% of the total detectable GDGTs as crenarchaeol (Dixie Valley and Seven Devils) (Table 1), and previous work showed that a third sample (40°C) contained crenarchaeol in 22:1 ratio to GDGT II (19). The abundance of crenarchaeol decreased to the 40 to 60% threshold between 50 and 65°C, causing the GDGT profiles of these hot springs (Fig. 1a) to resemble the GDGT distributions in marine sediments (Fig. 1b). GDGTs from the 87°C spring in Surprise Valley [samples SV (0 to 1 cm) and SV (1 to 2 cm) (Table 1)] are noticeably different (Fig. 1c) but still contain detectable traces of crenarchaeol (Table 1). When the abundance of crenarchaeol (I) is normalized to GDGT II (which has no temperature correlation) and plotted as a function of temperature, the data from terrestrial and marine systems together fit a second-order polynomial with significant correlation ($R^2 = 0.70$) (Fig. 2b). These results suggest that the marine archaea live on the low-temperature biosynthetic end of what may be a normal distribution centered around 40 to 45°C (Fig. 2b). Indeed, sediments underlying cooler marine waters always appear to contain relatively smaller amounts of crenarchaeol than samples obtained from tropical latitudes (24) (Table 2 and Fig. 2b). Most importantly, however, the

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TABLE 1. Data for hot springs in United States and Thailand, including relative abundance of GDGTs I to VII as determined by liquid chromatography-mass spectrometry

Spring	GPS data		Temp (°C)	pH	Relative abundance						
	North	East			II (*m/z* 1,302)	III (*m/z* 1,300)	IV (*m/z* 1,298)	V (*m/z* 1,296)	VII[a] (*m/z* 1,294)	I (*m/z* 1,292)	VI (*m/z* 1,292)
Buffalo Valley-1	4468421.0	472285.0	59.3	6.9	0.18	0.10	0.08	0.01	0.00	0.55	0.08
Buffalo Valley-2	4468421.0	472285.0	67.8	7.1	0.10	0.06	0.11	0.05	0.06	0.53	0.09
Buffalo Valley-3	4468421.0	472285.0	55.4	7.0	0.28	0.12	0.13	0.05	0.00	0.36	0.06
Dixie Valley	4405819.0	408469.0	45.2	9.2	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Fly Ranch	4525746.2	303453.8	48.6	8.5	0.30	0.05	0.04	0.00	0.03	0.54	0.04
Hard to Find-1	4540865.0	330711.7	85.7	7.0	0.09	0.10	0.13	0.19	0.30	0.02	0.17
Hard to Find-2[b]	4540865.0	330711.7	58.1	6.2	0.26	0.11	0.13	0.10	0.13	0.24	0.05
Jackson Mountain	4545834.3	355528.6	60.9	7.0	0.16	0.05	0.05	0.03	0.00	0.65	0.06
Paradise Valley	4585706.2	467623.8	52.6	6.8	0.10	0.02	0.01	0.01	0.00	0.73	0.13
Rick's Hot Spring	4504013.6	299900.7	57.9	7.3	0.07	0.03	0.02	0.02	0.04	0.70	0.12
Seven Devils	4437709.1	438123.5	36.5	7.9	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Soldier's Meadow	4581210.5	313942.0	48.0	8.6	0.22	0.02	0.04	0.06	0.06	0.52	0.08
SV-1 (Streamer)[c]	4602596.3	243657.9	86.5	5.8	0.39	0.34	0.17	0.07	0.03	0.00	0.00
SV-1 (0–1 cm)i[c]	4602596.3	243657.9	86.5	5.8	0.10	0.14	0.19	0.26	0.28	0.02	0.01
SV-1 (1–2 cm)[c]	4602596.3	243657.9	86.5	5.8	0.14	0.11	0.17	0.25	0.30	0.02	0.01
SV-2 (Vent wall)	4602702.2	243656.4	56.1	5.5	0.19	0.14	0.18	0.17	0.16	0.13	0.03
Thailand[d]	NA	NA	55.0	6.6	0.15	0.03	0.03	0.02	0.00	0.72	0.05

[a] The abundances of coeluting compounds I and VII were calculated from the fragment ion ratios of *m/z* 1,292 to 1,294, using a linear formula to account for the contribution of compound I to the 1,294 fragment. End members were the fragment ion ratios for authentic compound VII in a hyperthermophilic specimen containing no I and a cold-temperature marine sediment sample containing I but no VII.
[b] GDGT results were average values of two replicate analyses with an error of ±0.01 to ±0.07.
[c] Surprise Valley (SV) hot springs are located in California. The rest of the U.S. hot springs are located in Nevada. SV-1 (Streamer) was a white-streamer mat sample collected at the water surface; SV-1 (0–1 cm) and SV-1 (0–2 cm) were sediment samples collected at the edge of this hot spring.
[d] GPS data were not available (NA) for this site. Samples were collected at the Bor Khlueng hot spring in the Ratchaburi province of central Thailand.

data suggest that the optimum temperature for organisms that produce crenarchaeol is greater than either the present or past temperature of surface seawater.

It also is possible that pH, in addition to temperature, may affect the abundance of crenarchaeol in a manner similar to pH control of sterols in eukaryotes (9). These variables, however, cannot easily be decoupled for the organisms living in California or Nevada hot springs, as the lower-tem-

TABLE 2. Relative abundances of GDGTs in marine samples[a]

Marine sample	Location (sediment depth [cm])	Reference(s)	Temp (°C)	Relative abundance						
				II (*m/z* 1,302)	III (*m/z* 1,300)	IV (*m/z* 1,298)	V (*m/z* 1,296)	VII (*m/z* 1,294)	I (*m/z* 1,292)	VI (*m/z* 1,292)
a	Santa Monica Basin (0.75–1.5)	18	16	0.38	0.09	0.06	0.01	0.00	0.43	0.03
b	Santa Monica Basin (1.5–2.5)	18	16	0.35	0.09	0.06	0.01	0.00	0.47	0.02
c	Santa Barbara Basin (5.5–6.0)	18	16	0.35	0.06	0.03	0.01	0.00	0.53	0.02
d	Cariaco[b] (13.5–14.0)	17, 27	27	0.16	0.04	0.03	0.02	0.00	0.67	0.08
e	Black Sea	13, 24	14	0.45					0.42	
f	Arabian Sea	2	25	0.10					0.40	
g	Arabian Sea	2	24	0.19					0.57	
h	Arabian Sea	2	20	0.17					0.60	
i	Arabian Sea	2	24	0.14					0.64	
j	Arabian Sea	2	24	0.19					0.57	
k	Peru Margin	24, 28	20	0.36					0.46	
l	Skagerrak	24[c]	9	0.44					0.29	
m	Cariaco	17, 24	27	0.30					0.37	
n	Aegean Sea	6, 24	20	0.39					0.41	
o	Saanich Inlet	24[c]	10	0.56					0.34	
p	Kyllaren Fjord	24, 25	7	0.23					0.07	
q	Wadden Sea	24[d]	10	0.19					0.12	
r	Mok Bay	24[d]	11	0.29					0.21	

[a] GDGTs I and II were compiled from previous studies (samples e to r); and from GDGTs I to VII measured in this work (samples a to d). References for sea surface temperature (samples a to d) and both temperature and abundance (samples e to r) are given in column 3.
[b] Multicore 5, R/V *Hermano Gines*; 10°47.105′N, 64°42.868′W; May 2004.
[c] Annual mean surface water temperature for Saanich Inlet obtained from Fisheries and Oceans Canada (http://www-sci.pac.dfo-mpo.gc.ca/).
[d] Annual mean surface water temperature for North Sea regions from OSPAR Commission quarterly status report (http://www.ospar.org/eng/doc/pdfs/R2C2.pdf).

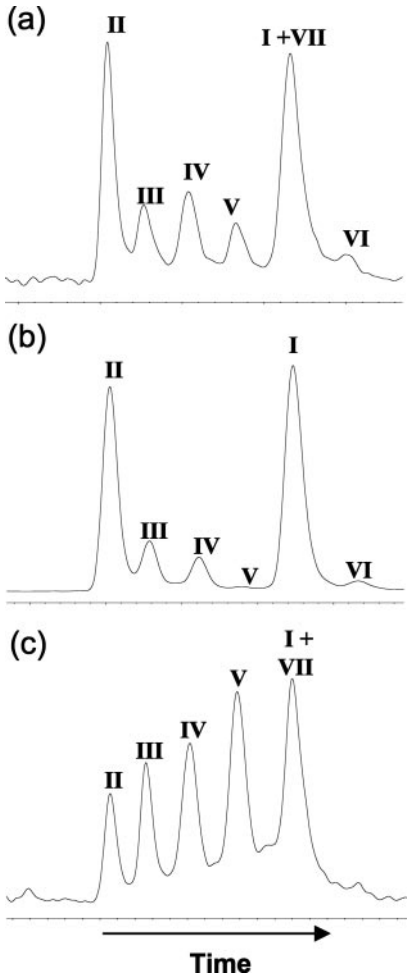

FIG. 1. Chromatograms of GDGTs from hot-spring and marine sediments. (a) Microbial mat from Nevada hot spring Hard To Find-2 (Table 1); 58.1°C; pH 6.2. (b) A typical marine sediment (Santa Monica Basin; sea surface temperature, 16°C). (c) Sediment from California hot spring Surprise Valley (SV-1); 86.5°C; pH 5.8. Compounds are identified using the roman numeral conventions (23) II, *m/z* 1,302, no rings; III, *m/z* 1,300, one cyclopentyl ring; IV, *m/z* 1,298, two cyclopentyl rings; V, *m/z* 1,296, three cyclopentyl rings; I, crenarchaeol, *m/z* 1,292, four cyclopentyl rings, one cyclohexyl ring; VI, *m/z* 1,292, crenarchaeol regioisomer; VII (new convention), *m/z* 1,294, four cyclopentyl rings. In panel a, I accounted for about 24% and VII accounted for about 13% of the total GDGTs, respectively; in panel c, traces of I (2%) were detectable, but VII dominated (28 to 30%) (Table 1).

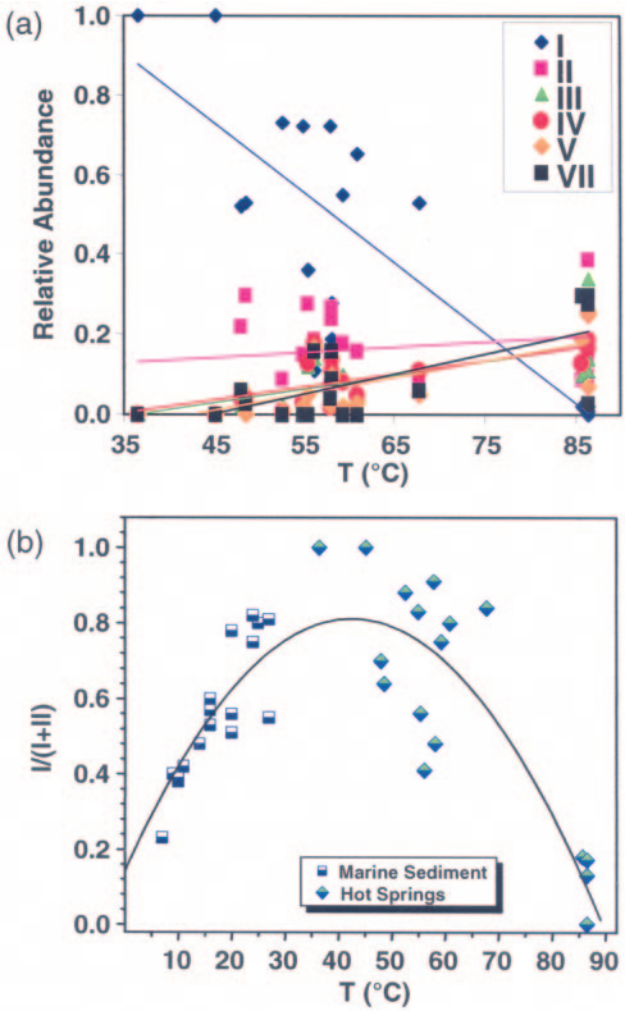

FIG. 2. Distribution of GDGTs as a function of temperature. (a) Relative abundances of GDGTs in hot-spring microbial mats between 36°C and 87°C. Linear trend lines are shown (R^2 values are reported in the text). Data for compound VI are not shown, as VI is always ~10% of the magnitude of I. (b) Ratio of I to II in samples from hot springs (Table 1) and marine sediments (Table 2) versus temperature. Polynomial curve fit; $y = -0.00037x^2 + 0.0315x + 0.141$; $R^2 = 0.70$.

perature springs tend to have higher pH values. A combined temperature-pH effect could explain the absence of the cyclohexyl ring in cultured thermophilic archaea, because these species commonly grow at low pH (26) rather than at the higher pH values measured here. Based on our findings and the observation that cyclohexyl rings have not been found in any *Euryarchaeota*, we hypothesize that crenarchaeol is a primitive phenotypic feature that is specific to the *Crenarchaeota*. While a temperature effect of the cyclohexyl ring has been reasonably explained (1), the effect of pH has not yet been rigorously examined.

Experimental studies using pure cultures of thermophilic archaea have demonstrated that the number of cyclopentyl rings in GDGTs increases with growth temperature (8, 26). Because the cyclohexyl ring has not been found in cultured thermophiles, its presence in the nonthermophilic *Crenarchaeota* has been attributed solely to the significant decrease in growth temperature experienced by the *Crenarchaeota* when they were colonizing marine waters, perhaps around 112 million years ago in the Cretaceous Period (1, 16). Our findings differ significantly from the view that crenarchaeol, or specifically, its unique cyclohexyl ring, is an evolutionary product of this relatively recent event (16, 22). The apparent ~40 to 45°C temperature optimum for crenarchaeol exceeds the warmest sea surface temperatures (27 to 36°C), even in the Cretaceous Period (22). We suggest that crenarchaeol could be an original and ancient biochemical property of the thermophilic *Crenarchaeota*, which occupy a deep branching point in the tree of life (3, 7).

We thank Christopher Romanek, Rick Socki, and Sue Lutz for help with field work in Nevada and Lily Eurwilaichitr for providing samples from Thailand. We thank Susan Carter and Sunita Shah for laboratory assistance. The owners of hot springs in Surprise Valley, Gerlach, and other locations showed great hospitality and supported our sampling efforts.

This research was supported by National Science Foundation grant MCB-0348180 to C.L.Z. and by NSF grant OCE-0241363 to A.P. The U.S. Department of Energy also supported this research through Financial Assistance Award DE-FC09-96SR18546 to the University of Georgia Research Foundation (C.L.Z. and G.M.).

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