Large Enantiomeric Excesses of L-Form Amino Acids in Deep-sea Hydrothermal Sub-vent of 156 °C Fluids at the Suiyo Seamount, Izu–Bonin Arc, Pacific Ocean

Yoshinori Takano,* Tsukasa Horiuchi,[†] Kensei Kobayashi,[†,††] Katsumi Marumo, and Tetsuro Urabe[†††]
Institute for Marine Resources and Environment, National Institute of Advanced Industrial Science and Technology, AIST Central 7, 1-1-1 Higashi, Tsukuba 305-8567
[†]*Department of Chemistry and Biotechnology, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501*
[††]*Institute of Space and Astronautical Science, 3-1-1 Yoshinodai, Sagamihara 229-8510*
[†††]*Department of Earth and Planetary Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033*

(Received June 19, 2003; CL-030554)

The present report describes the stereochemistry of amino acids under extreme conditions of 156 °C and greater than 140 atmospheres in a submarine volcanic hydrothermal sub-vent. The lack of evidence of abiotically synthesized amino acids such as ω-amino acid specimens and unexpected large enantiomeric excesses of L-form amino acids support the existence of a vigorous subjacent microbial oasis, which extends the known terrestrial habitable zone.

Deep-sea hydrothermal systems are suitable environments for chemical evolution and their role is implicated in the origins of life on earth.[1] Detection of anomalous dissolved glycine in the Red Sea also supports the notion of deep-sea chemical evolution.[2] A number of deep-sea biological colonies near black or clear smokers and the associated organic-rich seafloor microbial mats have been identified.[3] While the existence of a deep bacterial biosphere[4] in oceanic sediment has been reported and is a remarkable discovery, sub-vents or areas subjacent to seafloor hydrothermal vents are only recently being explored through deep-sea floor drilling experiments at submarine hydrothermal vents.[5]

Amino acids associated with living organisms, especially those found in proteins are only of the L-form, that is, a single enantiomeric form. The L-form proteinous amino acids are gradually converted to D-form amino acids, that is, racemization. Amino acids racemization primarily depends on the age and temperature of the sediment.[6] Here we report the first evaluation of the stereochemistry of D- and L-form amino acids under extreme conditions. The samples were collected as part of the Archaean Park Project[5] in a July 2001 cruise over the Suiyo seamount (28° 33 N, 140° 39 E)[7] in the Pacific Ocean. The deep-sea subterranean biosphere and geochemistry were examined by taking core samples at boring site APSK 07 (central hydrothermal venting area in the thermal active caldera floor) using a fixed seafloor benthic multicoring system (BMS) for pinpoint drilling in the large-scale submarine volcanic caldera (an area of approximately 1500 m × 500 m).[5] The following core profile was encountered (from top to bottom): dacite lava and/or pyroclastic rocks at about 1–3 m below the unconsolidated volcanic sands and pumice fragments; then a sheath, which acts as a cap rock of the terrestrial geothermal system comprised of clay minerals and anhydrite cement in addition to pyrite and other sulfide minerals; and end-member fluid ponding beneath the sheath. The degree of hydrothermal alteration within the volcanic rocks decreases at greater depths. Anhydrite was mainly precipitated from hot seawater as evidenced by the sulfur isotopic signature and the calcium/strontium ratio.[8,9] Sulfide grains composed mainly of pyrite, were observed at several zones, which may be the vestiges of hydrothermal veins. The maximum depth of the coring was 2690 mm below sea floor where the hydrothermal fluid temperature was measured at 156 °C by a custer-type thermometer.

Following suitable pretreatment of rock core samples, hydrolysed amino acids fractions were determined using ion-exchange high performance liquid chromatography.[10,11] Enantiomer separation for D/L ratio determination was accomplished by reverse-phase liquid chromatography.[11] The abundance of total hydrolysed amino acids in the core samples of APSK 07 ranged from 29.1 to 87.6 nmol/g-rock. Fresh internal samples of core rock contained mostly glycine with lesser concentrations of proteinous amino acids such as alanine, serine, and aspartic acid. Islam et al.[12] reported the predominant formation of ω-amino acid species such as γ-aminobutyric acid and δ-aminovaleic acid in Strecker synthesis of subcritical or supercritical hydrothermal fluids. Although nonproteinous amino acids such as β-alanine and γ-aminobutyric acid have been obtained as major products in laboratory experiments simulating hydrothermal systems,[12] they were present only as very minor constituents in the present sub-vent core samples of APSK 07 drilling site.

The D/L ratio of amino acids formed abiotically converges to approximately 1.0 in laboratory simulations.[13] However, the large enantiomeric excesses of L-form amino acids may indicate that the amino acids were observed at the hydrothermal surface and subterranean core samples. As shown in Table 1, the D/L

Table 1. Total hydrolyzed amino acids (THAA) and D/L ratio of aspartic acid (Asp), glutamic acid (Glu) and alanine (Ala) of hydrothermal sub-vent core samples in the site of APSK 07 at Suiyo seamount, Izu–bonin arc, Pacific ocean. Each column of THAA stands for the determination value as nmol/g-rock.

	D/L ratio			THAA
core	Asp	Glu	Ala	
1-01	0.32	0.10	0.01	87.6
1-03	0.23	0.13	0.01	34.8
1-04	0.10	0.07	0.24	40.6
2-01	0.50	0.22	0.01	67.1
2-03	0.25	0.17	0.04	51.7
3-03	0.17	0.10	0.01	36.5
3-05	0.13	0.10	0.02	29.1

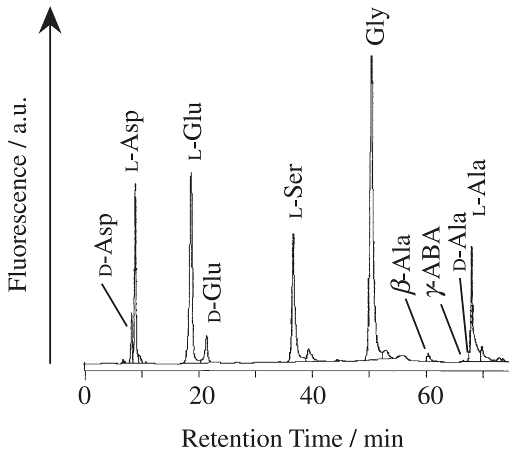

Figure 1. Separation of amino acid enantimers in deep-sea hydrothermal sub-vent core samples of APSK 07-1-01 at Suiyo seamount, Izu–bonin arc, Pacific ocean. Abbreviations; D,L-Asp: D,L-aspartic acid, D,L-Glu: D,L-glutamic acid, L-Ser: L-serine, Gly: glycine, β-Ala: β-alanine, D, L-Ala: D,L-alanine.

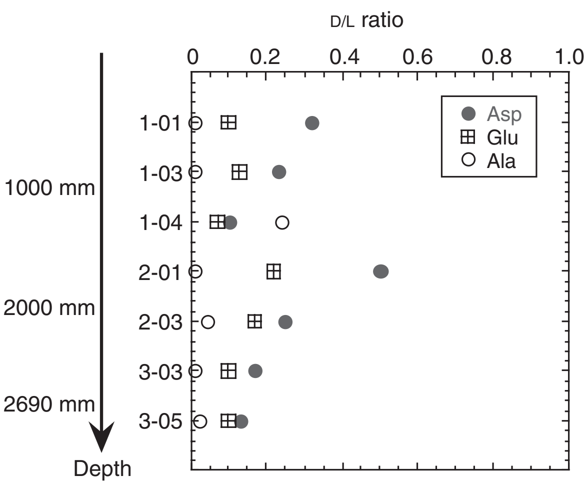

Figure 2. Vertical distribution of D/L ratios of aspartic acid (Asp,●), glutamic acid (Glu,⊞) and alanine (Ala,○) of hydrothermal sub-vent core samples in the site of APSK 07 at Suiyo seamount, Izu-bonin arc, Pacific ocean. The maximum depth of the coring was 2690 mm below sea floor.

ratio for aspartic acid in the volcanic sediment column was low (ranging from 0.10 to 0.50). Aspartic acid also had the fastest rate constant in the racemization reaction in this study. The order for the rate constant of racemization of aspartic acid, glutamic acid, alanine was in good agreement with literature values[14] except the case of the sample No. 1-04. The influence of the hydrothermal environment on the formation of L-form amino acids was clearly observed in the middle core (No. 2-01).

The vertical distribution of amino acids in these samples differs from that found in typical ocean seafloor sediment in that the concentration of amino acids in normal and/or simple sedimentation decreases rapidly with depth because of diagenesis.[15] Consequently, this investigation of sub-vent environment is essentially independent from surface conditions as its energy supply comes from chemical sources of fluids migrating upward from deeper levels in the sub-vent. Hence the large enantiomeric excesses of L-form amino acids detected in sub-vent samples were due to microbial influences. Preliminary investigation of the caldera revealed a high-temperature hydrothermal pool covered by several meters of volcanic sediment containing sulfate and topped by a boundary cap rock.[5] The currently accepted thermal limit of life is 113 °C.[16] Although some proteins from hyperthermophiles become more active at high pressure,[17] high pressure does not increase the thermal stability of micro molecules. It does appear to be difficult for life to exist at temperatures over 156 °C; however, the thermal gradient zone may be such that optimum temperatures for microbial life may be prevalent in sub-vent regions.

In conclusion, the low D/L ratio found lower in the column sediment may indicate that the prevailing thermal gradient in that area is optimal for subterranean microbes. The enantiomer ratios of the amino acids detected in sub-vents in the Suiyo seamount indicate a biogenic compounds rather than an abiotic chemical synthetic origin. The present findings provide significant evidence that previously unknown sub-vent systems are an extreme environment biosphere.

This research was funded by the Ministry of Education, Culture, Sports, Science and Technology of Japan through the Special Coordination Fund of the Archaean Park project; an international research project on interaction between the sub-vent biosphere and the geo-environment.

References

1 N. G. Holm, *Origins Life Evol. Biosphere*, **22**, 1 (1992).
2 D. E. Ingmanson and M. J. Dowler, *Nature*, **286**, 51 (1980).
3 T. Gold, *Proc. Natl. Acad. Sci., U.S.A.*, **89**, 6045 (1992).
4 R. J. Parkes, B. A. Cragg, S. J. Bale, J. M. Getliff, K. Goodman, P. A. Rochelle, J. C. Fry, A. J. Weightmann, and S. M. Harvey, *Nature*, **371**, 410 (1994).
5 T. Urabe, K. Marumo, K. Nakamura, M. Kinoshita, A. Maruyama, N. Seama, and J. Ishibashi, Japan Earth Planetary Science Joint Meeting, 2001, Abstr., No. Cm-001.
6 J. L. Bada and R. A. Schroeder, *Naturwissenschaften*, **62**, 71 (1975).
7 U. Tsunogai, J. Ishibashi, H. Wakita, T. Gamo, T. Masuzawa, T. Nakatsuka, Y. Nojiri, and T. Nakamura, *Earth Planet. Sci. Lett.*, **126**, 289 (1994).
8 K. Marumo, K. Ishii, and M. Noda, Japan Earth Planetary Science Joint Meeting, 2002, Abstr., No. B008-002.
9 T. Kakegawa, K. Marumo, and T. Urabe, Japan Earth Planetary Science Joint Meeting, 2002, Abstr. No. B008-003.
10 Y. Takano, H. Masuda, T. Kaneko, and K. Kobayashi, *Chem. Lett.*, **2002**, 986.
11 J. Kudo, Y. Takano, T. Kaneko, and K. Kobayashi, *Bunseki Kagaku*, **52**, 35 (2003).
12 M. N. Islam, T. Kaneko, and K. Kobayashi, *Anal. Sci.*, **17**, 1631 (2001).
13 H. Yanagawa and K. Kobayashi, *Origins Life Evol. Biosphere*, **22**, 147 (1992).
14 J. L. Bada and M. Y. Shou, "Biogeochemistry of amino acids," ed. by P. E. Hare, T. C. Hoering, and K. King, (1980), p 235.
15 E. Andersson, B. T. R. Simoneit, and N. G. Holm, *Appl. Geochem.*, **15**, 1169 (2000).
16 E. Bloch, R. Rachel, S. Burggraf, D. Hafenbradl, H. W. Jannasch, and K. O. Stetter, *Extremophiles*, **1**, 14 (1997).
17 G. Bernhardt, *Naturwissenschaften*, **71**, 583 (1984).

Published on the web (Advance View) September 22, 2003; DOI 10.1246/cl.2003.970