Amino acids in water samples from deep-sea hydrothermal vents at Suiyo Seamount, Izu-Bonin Arc, Pacific Ocean

Tsukasa Horiuchi¹, Yoshinori Takano^{1,2}, Jun-ichiro Ishibashi³, Katsumi
Marumo² Tetsuro Urabe⁴, and Kensei Kobayashi¹

¹ Department of Chemistry and Biotechnology, Yokohama National
University,

79-5 Tokiwadai Hodogaya-ku, Yokohama 240-8501, Japan

² Institute for Marine Resources and Environment, National Institute for
Advanced Industrial Science and Technology, AIST 7, 1-1-1 Higashi, Tsukuba,
Ibaraki 305-8567, Japan

³ Department of Earth and Planetary Science, Kyusyu University
Hakozaki Higashi-ku Hukuoka 812-8581, Japan

⁴ Department of Earth and Planetary Science, University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

*Corresponding author: Kensei Kobayashi

Department of Chemistry and Biotechnology, Yokohama National University,
79-5 Tokiwadai Hodogaya-ku, Yokohama 240-8501, Japan

Tel: +81-45-339-3938, Fax: Tel: +81-45-339-3938

(E-mail: kkensei@ynu.ac.jp)

Abstract

Pure seawater samples, at a temperature of 300°C (purity > 97%) were collected from deep-sea hydrothermal vents at Suiyo Seamount, Izu-Bonin Arc, Pacific Ocean as a part of the Archaean Park Project. Dissolved and total hydrolyzable amino acids were determined by ion-exchange HPLC, and for the first time, their enantiomeric ratios were measured by reversed-phase HPLC. Glycine and serine were the two most abundant amino acids, followed by other proteinaceous amino acids such as alanine, glutamic acid and aspartic acid. Non-proteinaceous amino acids, e.g. β -alanine and γ -aminobutyric acid, were detected as minor constituents. The majority of the amino acids detected were of the L-form which suggest most of the amino acids detected were formed biologically and that there are active microbial community near these hydrothermal systems.

Keywords: deep-sea hydrothermal vent, hydrothermal water, amino acids, subterranean biosphere

Introduction

The discovery of submarine hydrothermal systems (SHSs) in the late 1970's (Corliss et al., 1979) and extrathermophilic organisms living there has led researchers to consider SHS environments suitable for chemical evolution and for the origins of life on the Earth (Holm, 1992). Many efforts have been made to collect fluids from submarine hydrothermal vents, and the distribution of stable inorganic constituents and isotopic ratios ($\delta^{13}\text{C}$, etc.) of SHSs have been well documented.

Amino acids are essential organic molecules for life. Proteins, which are one of the most important biopolymers, are polycondensation products of 20 different amino acids. With the exception of glycine all proteinaceous amino acids are optically active. L-enantiomers are far more common than D-enantiomers in living systems, although D-amino acids do exist as minor constituents of bacterial cell walls and in peptide antibiotics (Lee et al., 1977) and more recent work states that bacterial biomass is rich in D-amino acids (Dittmar et al., 2001). Amino acids found in environments of low biological activity show higher D/L ratios. Thus the D/L ratio of amino acids could be used as a biomarker to verify microbial activity in hydrothermal environments.

In 1953, Miller reported abiotic formation of amino acids from a mixture of methane, ammonia, hydrogen and water by spark discharges. There have been several studies to synthesize amino acids in simulated submarine hydrothermal systems (Yanagawa and Kobayashi, 1992; Hennessey et al., 1992; Marshall, 1994; Islam et al., 2001). Ingmanson and Dowler (1980) reported

that a high concentration of glycine was observed in seawater sampled near submarine hydrothermal vents of the Red Sea. These results suggest that amino acids may be formed abiotically in submarine hydrothermal systems. The D/L ratio of abiotically formed amino acids is 1, which is a racemic mixture. Proteinaceous amino acids and non-proteinaceous amino acids were found among the major products of abiotic synthesis.

Thus, the composition and D/L ratio of amino acids can be a good indicator for the origin and history of organic molecules - i.e., abiogenesis and biogenesis. There have been only a few attempts to analyze the composition and D/L ratio of amino acids in the brine of SHSs. It is indicated that there have been attempts to analyze AA composition and DL ratios but no reference were provided for D/L ratios or the conclusions that previous workers have reached. This paper presents the results of the composition and enantiomeric ratio of amino acids in hydrothermal water samples directly collected from deep-sea hydrothermal systems at the Suiyo Seamount, Izu-Bonin Arc, Pacific Ocean.

Geological setting and hydrothermal fluid discharges

The Izu-Bonin (Ogasawara) Arc lies on the eastern rim of the Philippine Sea plate. This arc is about 1,200 km long and extends from the Izu Peninsula (35 °N, 139 °E) to Minami-Iwojima Island (24 °N, 141°E). This arc is part of the circum-Pacific island arc system, and is adjacent to the Northeast Japan Arc and the Mariana Arc to the north and south respectively. Many volcanic islands and submarine volcanoes run parallel to the Izu-Bonin trench

and form the volcanic front of this intra-oceanic island-arc system. The southern Izu-Bonin Arc, which is divided by the Sofugan tectonic line from the northern Izu-Bonin Arc (Tsunogai et al., 1994) (Figure 1-a), is thought to have started its activity 42 Ma ago (Yuasa, 1992). The Shichiyo seamount chain forms a volcanic front (Figure 1-b) around which the arc crust is inferred to become thinner than that in the northern part (Hino, 1991). The Suiyo Seamount, one of the volcanoes in the Shichiyo chain, has two major peaks: one each on its eastern and western sides. Dacite rocks of a calc-alkaline rock series and low-potassium andesites were recovered (Watanabe and Kajimura, 1993). Seafloor hydrothermal alteration at Suiyo from the point of view of geochemical and mineralogical characteristics were preliminary reported (Marumo et al., 2002, 2003).

Experimental

Sampling

In the caldera of Suiyo seamount, intense hydrothermal activity has been observed through the existence of many hydrothermal vents. Hydrothermal chemical components of end members showed that the various temperatures of hydrothermal fluids arise from dilution by surrounding seawater (Sunamura et al., 2003). Water samples were collected as a part of the Archaean Park Project onboard R/V Shinryu-Maru over the Suiyo Seamount (28°33 N, 140°39 E) in August, 2002. Hydrothermal water samples from natural vents and those from artificially-drilled vents were collected at a depth of 1380 m by the remotely operated vehicle (ROV) *Hakuyo* 2000 (Shin Nihon Kaiji Ltd.) Surrounding seawater samples were taken using Niskin

bottles at a depth of 1370 m. Sampling sites are shown in Table 1. The purity of hydrothermal waters was evaluated from the concentration of magnesium (Von Damm, et al.,1985).

Seawater sample was filtered through cellulose acetate membrane filters of 0.2µm pore size immediately after collection. Both filtered and non-filtered fractions were stored frozen until analysis in the laboratory. The former was used to determine total dissolved hydrolyzed amino acids (TDHAA), and the latter was used to determine total hydrolyzed amino acids (THAA).

Determination of amino acids

A subsample of 20 ml was dried *in vacuo* in a test tube with a diaphragm pump. 6 M HCl was added to each of the test tubes. The test tubes were sealed, placed in a block heater, and heated at 110°C for 24 hours. The hydrolysates were then dried *in vacuo* again, followed by desalting with Bio-Rad AG-50W-X8 cation-exchange resin column (200-400 mesh). Before application of the sample, the resin was cleaned by passing 1M HCl, H₂O, 1M NaOH and H₂O successively through the column. Just before applying the sample the resin was reactivated with 1M HCl and rinsed with H₂O. The amino acid fraction was obtained by eluting with 10% NH₃. The eluate was dried *in vacuo*, and 1mM HCl was added to each of the test tubes. Hydrophobic impurities in the solution were removed by solid phase extraction with a TOYOPAK ODS. column. The eluate was dried *in vacuo* again, and re-dissolved in water (1ml).

The concentration of THAA and TDHAA were determined by a

cation-exchange HPLC system (two Shimadzu LC-6A pumps, a Shim-pack ISC-07/S1504 column (sodium type 4.0 mm i. D $\times$ 150 mm), a Shimadzu RF-535 fluorometric detector, and a post-column derivatization system with o-phthalaldehyde (OPA) and N-acetyl-L-cysteine (N-AcCys)). Analytical conditions are reported in a previous publication. (Kobayashi et al., 1991).

Separation of amino acid enantiomers

The determination of amino acid enantiomers was achieved with a RP-HPLC system, which was composed of high performance liquid chromatograph pumps (TOSOH CCPM II), a reversed-phase column (YMC-pack Pro C18 4.6 mm i.d. $\times$ 250mm) and a TOSOH FS-8020 fluorometric detector (excitation wavelength : 355nm ; emission wavelength : 435nm). An aliquot of the desalted amino acid fraction was mixed with OPA and N-AcCys in a glass vial. The derivatives were purified by solid phase extraction column TOYOPACK-ODS cartridge to eliminate hydrophobic impurities. Average recovery of amino acids after the solid phase extraction was ca. 94 %. The eluent was injected into the RP-HPLC system. Gradient elution was applied by using the following eluents; A: 40 mM sodium acetic acid buffer (pH 6.5), B: 100% methanol (ultra-pure HPLC grade). Gradient program was performed as follows: 10 min (Eluent B: 0 %) – 25 min (Eluent B: 10 %) – 65 min (Eluent B: 20 %) – 80 min (Eluent B: 20 %) – 85 min (Eluent B: 40 %) – 115 min (Eluent B: 60 %) – 120 min (Eluent B: 80 %) – 135 min (Eluent B: 0 %) (Takano et al., 2003).

All glassware was heated in a high temperature oven (Yamato DR-22) at

500°C prior to use in order to eliminate any possible contaminants. Water was purified successively with a Milli-Q Labo and Simpli Lab- UV (both Milli-pore Corporation).

Results and discussion

Amino acid composition in the water samples

We determined THAA (non-filtered portion) and TDHAA (filtered portion) concentrations in 7 seawater samples from the hydrothermal vents and 1 seawater sample from the surrounding environment (listed in Table 1). In a typical HPLC chromatogram of THAA, glycine was predominant, followed by serine. Major peaks can be assigned to proteinaceous amino acids. Small peaks of non-proteinaceous amino acids such as β -alanine and γ -aminobutyric acid were also detected. Procedural blank analyses were performed by using the same water, glassware, ion-exchange resin and HPLC systems, which gave a trace amount (less than 10 pmol/ml) of glycine (Takano et al., 2003). Therefore, we determined amino acid concentrations in hydrothermal water and sea water samples from Suiyo Seamount.

The concentrations of THAA and TDHAA in the samples are summarized in Tables 2 and 3, respectively. TDHAA in sample No.4 could not be determined due sample size limitation. Temperature of the high-purity samples (No.6 and No.7) is ca. 300°C, and that of lower purity water is lower. Thus, it can be said that amino acid composition of sample No.6 and No.7 largely reflects that of the hydrothermal fluid itself. On the other hand, it seems that amino acid composition of the other samples with lower

temperature and lower purity (No.2, No.3 and No.4) reflected that of both high temperature fluid and surrounding seawater.

In all the samples examined, proteinaceous amino acids such as glycine and serine were more abundant than non-proteinaceous amino acids such as β -alanine and γ -aminobutyric acid. Ingmanson and Dowler (1980) reported anomalous amounts of glycine in Red Sea brine, and they suggested that a possible source of the amino acids was an abiotic origin. However, a similar anomaly was not observed in our samples. Among the proteinaceous amino acids, neutral amino acids were predominant followed by acidic amino acids. Aromatic and sulfur amino acids were found only as minor constituents.

THAA in the seven hydrothermal fluids ranged from 240 to 1160 nmol/L. If we assume that the collected samples were mixtures of actual hydrothermal fluids that had been erupted and seawater in the surrounding environment (No.8), the THAA of the hydrothermal fluids can be calculated. For example, the THAA of the sample No.6 and No.7 (the two purest samples) was estimated as 900 nmol/L and 568 nmol/L, respectively, by using the mixing ratio of the hydrothermal fluid and the surround seawater (No.8) obtained from magnesium concentration (Von Damm, et al., 1985). THAA concentration in those samples was similar to concentration in the Okinawa Trough submarine hydrothermal vent fluid reported by Kobayashi et al. (1995). It is lower by one order of magnitude than THAA in interstitial waters sampled from Izu-Bonin Island Arc. (Kawahata et al.,1992).

THAA should be more than TDHAA, and this was observed in most samples. Only in the case of No.6, THAA obtained is more than TDHAA.

On the other hand, THAA in No.6 is much higher than No.7. These can be explained as follows: sample No.7 and sample No.6 are the purest black smoker fluids with large concentration of hydrogen sulfide and inorganic particles like FeS. Absorption of amino acids to sulfide particles caused the nonuniformity of the sample, which may be the reason of the abnormalities of sample No.6 and sample No.7 described above.

Enantiomeric ratios of amino acids

A reversed-phase (RP) HPLC chromatogram of THAA in sample No.6 is shown in Fig. 2.

The enantiomeric ratios for hydrothermal water and marine water are shown in Table 4. The L-enantiomer is present in much greater quantities for Ala, Asp and Glu, than the D-enantiomer.

Amino acids of biological origin are basically L-form, although D-glutamic acid, D-alanine, and D-aspartic acid have previously been reported in the cell walls and capsules of bacteria and some D-amino acids have been found in the extracellular waste products of bacteria (Lee et al., 1977). D-Amino acids are also formed by racemization of L-amino acids, particularly at high temperature, so that chemical racemization of amino acids can be a major source of D-amino acids over geological time (Bada et al., 1975). Thus the quite small values of D/L ratio for amino acids observed in this study indicate that amino acids in hydrothermal samples were mostly of biological origin, and that only limited racemization / chemical transformation has occurred.

Abiotic formation of amino acids?

Since the pioneering research of Miller (1953), numerous laboratory experiments concerning the formation of biologically interesting organic compounds have been undertaken. It has been also reported that amino acids can be formed abiotically in simulated submarine hydrothermal environments. For example, amino acids were detected in the product of Strecker-type reactions in the super critical hydrothermal water flow condition simulating submarine hydrothermal systems (Islam et al., 2001). Non-proteinaceous ω -amino acids such as β -alanine, γ -amino butyric acid and δ -amino valeric acid were found among the major amino acid products in the flow reactor at high temperature and high pressure (Islam et al., 2001). Ingmanson and Dowler (1980) reported that an extraordinary predominance of glycine was observed in Red Sea brine, and suggested that is an evidence for the abiotic formation of amino acids in submarine hydrothermal systems. ω -Amino acids were detected in some samples e.g. sample No.6, No.2, but they are only minor constituents in concentration. In addition, D/L ratio of the amino acids observed was quite low, while that of abiotically-formed amino acids is nearly 1. Thus we conclude that most of amino acids found in the present hydrothermal fluids are not abiotically formed, but of biological origin.

Conclusions

High purity, hydrothermal fluids were sampled from natural and drilled vents in hydrothermal systems at Suiyo Seamount, Izu-Bonin Arc, Pacific

Ocean. The results presented suggest that there are biological sources of amino acids near submarine hydrothermal sub-vent systems. It agrees with the results that approximately 10^4 - 10^5 cell/ml of microbes were found in hydrothermal fluid from the drill hole at Suiyo hydrothermal area (Maruyama et al., 2001). These results indicate that would extend the terrestrial habitable zone in extended further than previously known.

Acknowledgements

The authors thank to Prof. J. Ishibashi , chief scientist of the Shinryu-Maru cruise and the other scientists, ROV operating team and crew of the cruise. Also, We are grateful to Mr. T. Kaneko, Yokohama National University, for providing technical advice and discussion. 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

- Bada, J. L., Schroeder, R. A., 1975. Amino acid racemization reactions and their geochemical implications. *Naturwissenschaften* 62, 71-79.
- Corliss, J. B., Dymond, J., Gordon, L. I., Edmond, J. M., von Herzen, R. P., Ballard, R. D., Green, K. K., William, D., Bainbridge, A., Crane, K., van Andel, T. H., 1979. Submarine thermal springs on the Galapagos Rift.

- Science. 203, 1073-1083.
- Dittmar, T., Fitznar, H. P., Kattner, G., 2001. Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. *Geochimica et Cosmochimica Acta* 65, 4103-4114.
- Hennet, R. J. C., Holm, N. G., and Engwl, M. H., 1992. Abiotic synthesis of amino acids under hydrothermal conditions and the origin of life *Naturwissenschaften*. 79, 361-365.
- Hino R., 1991. Crustal structure below the sea-floor around the Japan Islands. *J.Geograph*. 100, 583-595. (in Japanese with English Abstract).
- Holm, N. G. (Ed.). 1992. Marine hydrothermal systems and the origin of life-special issue. *Origins Life Evol. Biosphere*. 22, 1-242.
- Ingmanson, D. E. and Dowler, M. J., 1980. Unique amino acid composition of Red Sea 20 brine. *Nature*. 286, 51-52.
- Islam, M. N., Kaneko, T., Kobayashi, K., 2001. Determination of amino acids formed in a supercritical water flow reactor simulating submarine hydrothermal systems. *Analytical Sciences* 17, 1631-1634.
- Kawahata H., Ishizuka T., (1992) Amino acids in interstitial waters from sites 790 and 791 in the Izu-Bonin island arc Taylor B.,Fujioka K., et al., *Proceedings of ocean drilling program. Scientific results. vol.126*
- Kobayashi, K., Kohara, M., Gamo, T., Yanagawa, H., 1995. Formation and alternation of organic compounds in simulated submarine hydrothermal vent environments. *Bioorganic processes and the Ocean Flux in the Western Pacific*. 523-535
- Kobayashi, K., Kaneko, T., Kobayashi, T., Hui, L., Tsuchiya, M., Saito, T.,

- Oshima, T., 1991. Analysis of products synthesized from simulated primitive planetary atmospheres. I. Amino Acids. *Analytical Science* 7, 921-924.
- Kudo, J., Takano, Y., Kaneko, T., Kobayashi, K., 2003. A pre-treatment method for determination of amino acids and their D/L ratios in soil samples. *Bunseki Kagaku*. 52, 35-40. (in Japanese with English Abstract)
- Lee, C., Bada, J. L., 1977. Dissolved amino acids in the equatorial Pacific, the Sargasso Sea, and Biscayne Bay. *Limnology and Oceanography* 14, 740-747.
- Marshall, W. L., 1994. Hydrothermal synthesis of amino acids. *Geochimica et Cosmochimica Acta* 58, 2099-2106.
- Marumo, K., Ishii, K., Noda, M., 2002. Seafloor hydrothermal alteration at Suiyo submarine volcano: geochemical and mineralogical characteristics. Japan Earth and Planetary Science Joint Meeting, B008-002.
- Marumo, K., Urabe, T., and Nakashima, M., 2003. Geochemistry and mineralogy of the hydrothermal system at Suiyo Seamount. Japan Earth and Planetary Science Joint Meeting, B002-001.
- Maruyama, A., Sunamura, M., Higashi, Y., Ishibashi, J., Kakegawa, T., 2001. Preliminary report for NT 01-09 cruise, Proceeding for JAMSTEC 18th Shinkai Symposium. p.44 (in Japanese).
- Miller, S. L., 1953. Production of amino acids under possible primitive earth condition. *Science*. 117, 528-529.
- Takano, Y., Horiuchi, T., Kobayashi, K., Marumo, K., Urabe, T., 2003. 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. *Chemistry Letters* 32, 970-971.
- Takano, Y., Sato, R., Kaneko, T., Kobayashi, K., Marumo, K., 2003. Biological origin for amino acids in a deep subterranean hydrothermal vent, Toyoha mine, Hokkaido, Japan. *Organic Geochemistry* 34, 1491-1496.
- Tsunogai, U., Ishibashi, J., Wakita, H., Gamo, T., Watanabe, K., Kijimura, T., Kanayama, S., Sakai, H., 1994. Peculiar features of Suiyo Seamount hydrothermal fluids Izu-Bonin Arc: Differences from subaerial volcanism. *Earth and Planetary Science Letters* 126, 289-301.
- Von Damm, K.L., Edmond, J.M., Grant, B., Measures, C.I., Walden, B., Weiss, R.F., 1985. Chemistry of submarine hydrothermal solutions at 21° N, East Pacific Rise. *Geochimica et Cosmochimica Acta* 49, 2197-2220.
- Watanabe, K., Kijimura, T., 1993. Topography, geology and hydrothermal deposits at Suiyo Seamount, Proc. JAMSTEC Symp. Deep-Sea Res. Special Issue. 9, 77-89. (in Japanese with English Abstract)
- Yanagawa, H., Kobayashi, K., 1992. An experimental approach to chemical evolution in submarine hydrothermal systems. *Origins of Life and Evolution of the Biosphere* 22, 147-159.
- Yuasa, M., 1992. Origin of along-arc variations on the volcanic front of the Izu-Ogasawara-Bonin Arc. *Bulletin Geological Survey of Japan* 43, 457-466.

FIGURE CAPTIONS

Fig. 1 (a) Geological location of the Izu-Bonin Arc on the eastern edge of the Philippine Sea plate, western Pacific ocean. (b) Topographic map of the Suiyo seamount in the Shichiyo seamount chain cited from Tsunogai et al., 1994. *Si* = Suiyo Seamount; *OR* = Ogasawara Ridge; *OT* = Ogasawara Trough; *S* = Sofugan Island; *N* = Nichiyo Seamount; *G* = Getsuyo Smt.; *K* = Kayo Smt.; *M* = Mokuyo Smt.; *Kn* = Kinyo Smt.; *D* = Doyo Smt.; *Ns* = Nishinoshima Island.

Fig.2 Reverse-phase chromatogram of hydrolyzed amino acids in the hydrothermal water of No. 6 sample . Abbreviations for amino acids: D, L-Asp: D, L-aspartic acid, Ser: serine, D, L-Glu: D, L-glutamic acid, D, L-Ala: D,L-alanine, β -Ala: β -Alanine. D- and L- serine peaks are not separated.