ELSEVIER

Thermochimica Acta 375 (2001) 9–16

thermochimica acta

www.elsevier.com/locate/tca

Kinetics of the racemization of amino acids at 225–275°C using a real-time monitoring method of hydrothermal reactions

Kunio Kawamura*, Masaru Yukioka

Department of Applied Chemistry, Osaka Prefecture University, Gakuen-cho 1-1, Sakai, Osaka 599-8531, Japan

Received 18 December 2000; received in revised form 12 March 2001; accepted 12 March 2001

Abstract

The monitoring method of rapid hydrothermal reactions was successfully applied to the racemization of amino acids at 225–275°C. The rate constants of racemization of alanine, leucine, and phenylalanine were determined and the apparent activation energy was 124 kJ mol^{-1} for alanine, 120 kJ mol^{-1} for leucine, and 115 kJ mol^{-1} for phenylalanine, which are in good agreement with previous data at lower temperatures. In the course of the study, the observation of the formation of alanine anhydride, which is not possible by the conventional batch method, has been succeeded. Further, the cyclization of L-alanyl-L-alanine was possible to be monitored and it was confirmed that the reaction was much faster than racemization of alanine. Basing on the kinetic investigations, the importance of the racemization of amino acids is discussed from the viewpoint of the hydrothermal origin of life.

Keywords: Racemization of amino acids; Hydrothermal reaction; Flow method for reaction kinetics; Hydrothermal origin of life

1. Introduction

It is widely believed that hydrothermal systems such as hydrothermal vents in deep ocean have played important roles for the emergence of life under the primitive earth conditions (the hypothesis on the hydrothermal origin of life) [1,2]. For instance, there have been successful studies, which demonstrate the formation of biologically important molecules such as proteins under the hydrothermal conditions [3–7]. In addition, phylogenetic analyses indicate that the last common ancestor of all present organisms had the nature of hyperthermophiles [8–11]. Besides, the rapid degradation of biomolecules is considered as a stumbling block in the hypothesis on the hydrothermal origin of life [12–15]. For instance, the low stability of RNA at high-temperatures is in conflict with the RNA world hypothesis [16–22]. However, during investigations the detailed kinetics of the degradation and formation of these molecules under hydrothermal environments, we have been confronted with difficulty on monitoring the reactions at high-temperatures. Thus, as one solution, we have recently proposed a new monitoring method of hydrothermal reactions [23–25].

On the other hand, the racemization and the amplification of asymmetric compounds are interesting from the viewpoint of emergence of chiral molecules at the time of origin of life. For instance, the rapid racemization of amino acids at high-temperatures would have forbidden the emergence of advanced

* Corresponding author. Tel.: +81-722-52-1161; fax: +81-722-54-9903.
E-mail address: kawamura@chem.osakafu-u.ac.jp (K. Kawamura).

0040-6031/01/$ – see front matter
PII: S0040-6031(01)00521-4

functions as being observed in the modern organisms. Further, the racemization is useful in geochronology [26,27]. However, real-time monitoring the racemization of amino acids over 200°C is difficult or impossible, then the rate constants at high-temperatures have been exclusively obtained by extrapolation of the Arrhenius plots determined at lower temperatures [28–31]. In the present study, the scope of the real-time monitoring method was investigated for the racemization of amino acids, then monitoring the racemization of alanine, leucine, and phenylalanine has been succeeded at 225–275°C at 10 MPa. Further, the condensation of two alanine molecules to form alanine anhydride was possible to be followed during racemization, while no oligopeptide was found. The kinetic analysis demonstrates the usefulness of the monitoring method and the importance of the racemization of amino acids is discussed from the viewpoint of the hydrothermal origin of life.

2. Experimental

2.1. Chemicals

L-Alanine was purchased from Peptide Institute Inc. (Osaka, Japan) and alanine anhydride (mixture of DL and meso 3,6-dimethyl-2,5-piperazinedione), L-alanyl-L-alanine, and D,L-,D,L-alanylalanine were purchased from Sigma–Aldrich Japan (Tokyo, Japan). All other reagents used were obtained from Sigma (St. Louis, MO, USA) and Wako (Osaka, Japan).

2.2. Apparatus

The monitoring system for hydrothermal reactions was set up as described in previous studies (Fig. 1) [24,25]. The system consists of a 1 l H_2O reservoir, a high-pressure HPLC pump (model PU-980, JASCO Corporation, Japan), a sample injector (Rheodyne, with 0.1 ml sample loop), a hydrothermal reactor, a cooling bath, and a back-pressure tubing, sampling port, and a temperature controller. The system was connected with transfer lines (0.1 or 0.25 mm inner diameter (i.d.) of stainless steel (SUS) tubing), and double-distilled water, was filtered with a 0.2 μm membrane filter, was pumped from the reservoir against the back-pressure on the system. The pressure in the reactor was regulated using back-pressure tubing at 10 MPa. The flow reactor consisted of tubing and a heating block, which the temperature was controlled at ±1%. The residence time in tubing was controlled by changing the flow rate of the pump at 0.05–2 ml min^{-1} and using tubing with different sizes. The inner volumes of 0.1 and 0.25 tubing mm i.d. tubing were determined by weighing with and without filling water at 25°C.

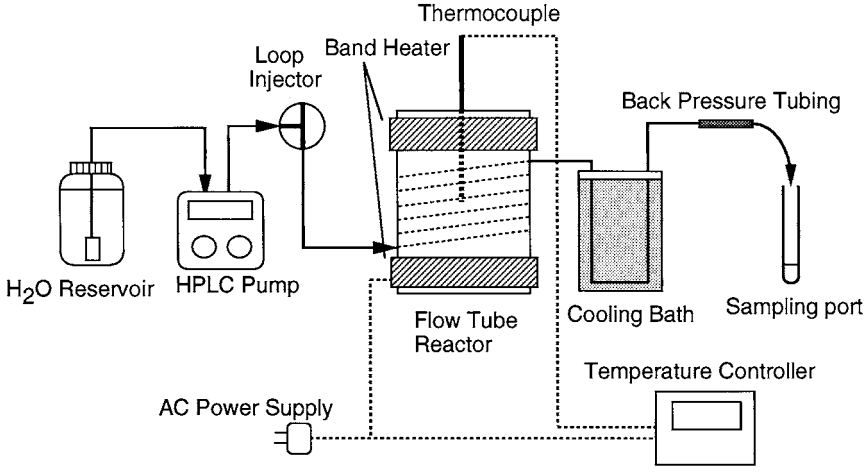

Fig. 1. Hydrothermal system: the system consists of a H_2O reservoir, a high-pressure pump, a sample injector, a hydrothermal reactor, an ice bath, a back-pressure equipment and sampling port, and a temperature controller.

2.3. Sample preparation and kinetic analysis

Sample solutions containing 0.01–0.5 M amino acid, 0.1 M NaCl, 0.05 M $MgCl_2$ were prepared and pH was adjusted with 0.01 M HCl or 0.01 M NaOH solutions. An amount of 0.1 ml of the sample solution was injected to the flow system and the sample exposed at a high-temperature was withdrawn from the sampling port at an appropriate time period after the sample injection and immediately quenched to 0°C. The sample was analyzed by HPLC (Shimadzu HPLC LC-10A, Kyoto, Japan) with a chiral separation column CROWNPAK CR(+) (Daicel Chemical Industries Ltd., Tokyo, Japan). The HPLC analysis was carried out at 25°C and a wavelength 220 nm was used for detection.

A computer program SIMFIT was used for evaluation of the rate constants [32].

3. Results and discussion

3.1. Racemization of amino acids

HPLC charts of the products of racemization of L-alanine at 275°C are shown in Fig. 2. D-Alanine and alanine anhydride were assigned using authentic samples, and unknown peaks were also detected. The reaction curves of racemization of alanine were successfully determined at 225–275°C and 0.02–0.5 M alanine using the present system (Fig. 3). Besides, in order to see if the tubing material affects the racemization, polyetheretherketone (PEEK) tubing was tested at 225°C. The reaction curves using SUS and PEEK tubing were in good agreement, so no influence of the inner wall of SUS and PEEK tubing was confirmed. Thus, further investigations were performed using SUS tubing since SUS tubing bears at higher temperatures than PEEK tubing. The extents of the products of racemization from L-alanine to D-alanine and its reverse reaction were in good agreement at the end of the reaction (Fig. 3a and b). Besides, the formation of alanine anhydride isomers was less pronounced at low concentrations of alanine (Fig. 2a and b). Although, the reaction curves indicate that the HPLC peaks of L-alanine and isomers of alanine anhydride are somewhat overlapping (Figs. 2c and 3a and b), it was possible to determine the rate constants of the

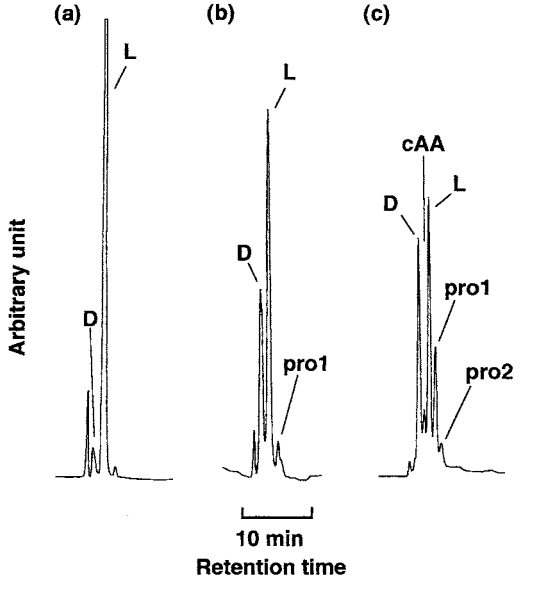

Fig. 2. HPLC chromatograms for the racemization of L-alanine. The reactions were carried out in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$ at initial pH = 7.0 at 275°C. (a) 0.05 M L-alanine, residence time: 2.9 s. (b) 0.05 M L-alanine, residence time: 58.1 s. (c) 0.2 M L-alanine, residence time: 116 s. Abbreviations: L, L-alanine; D, D-alanine; cAA, alanine anhydride; pro1, unknown product 1; pro2, unknown product 2.

racemization and the formation of alanine anhydride. The racemization of alanine is described by following equations.

$$\text{L-alanin} \rightleftharpoons \text{D-alanine} \tag{1}$$

$$-\frac{\mathrm{d}}{\mathrm{d}t}[\text{L-alanine}] = k_{\text{rac}}[\text{L-alanine}] - k_{\text{rac}}[\text{D-alanine}] \tag{2}$$

$$\ln\left\{\frac{(1+[\text{D}]/[\text{L}])}{(1-[\text{D}]/[\text{L}])}\right\} = 2k_{\text{rac}}t \tag{3}$$

where k_{rac} is the rate constant of racemization, and [D] and [L] indicate D-amino acid and L-amino acid, respectively. The first-order rate plots expressed by Eq. (3) demonstrate that the racemization of alanine obeys the first-order rate kinetics at low concentration of alanine (Fig. 4) since the yield of alanine anhydride isomers is low (Fig. 2a and b).

By conventional batch methods, real-time monitoring the formation of alanine anhydride is not possible because of rapid cyclization of alanylalanine at high-temperatures [13]. However, the formation of alanine

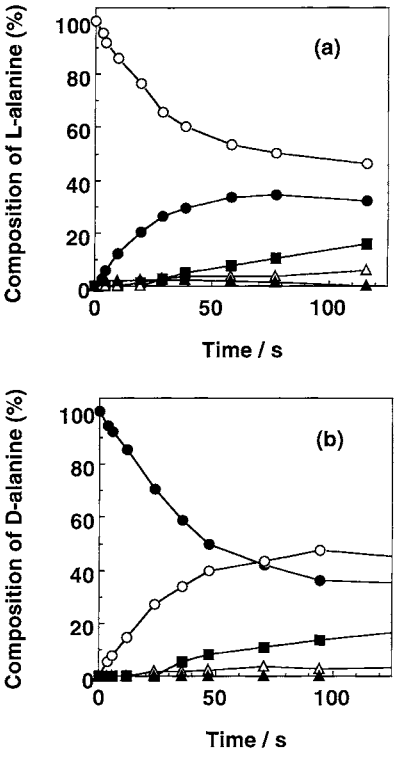

Fig. 3. The reaction curves for the racemization of L-alanine and D-alanine. The reactions were carried out in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.5 M L- or D-alanine at initial pH = 7.0 at 275°C. (a) L-alanine, (b) D-alanine. (○) L-alanine + alanine anhydride isomer; (●) D-alanine; (■) alanine anhydride isomer; (△) unknown product 1; (▲) unknown product 2.

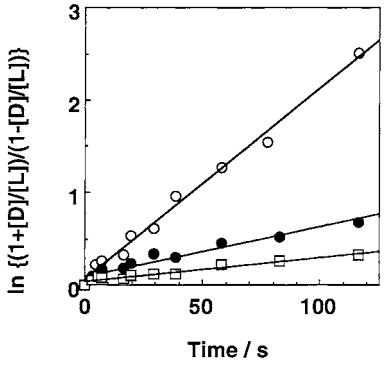

Fig. 4. The first-order rate plots for the racemization of L-alanine. The reactions were carried out in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.05 M L-alanine at initial pH = 7.0 at 275°C. (○) 275°C; (●) 250°C; (□) 225°C.

anhydride was possible to be followed by the present method. The extent of alanine anhydride isomers increases with increasing the alanine concentration and the reaction temperature, and the extent was not significant at 225 and 250°C (Fig. 2a and b). This fact is consistent with previous studies, in which the yield of diketopiperadine formed from glycine increases with increasing temperature [7,13,33]. In addition, the formation of dipeptide isomers from alanine monomer was not detected in the products. Thus, the formation of alanine anhydride is expressed by Eq. (4)

$$\text{Alanine} + \text{alanine} \underset{k_{pip}}{\rightarrow} \text{alanine anhydride} \quad (4)$$

where k_{pip} indicates the rate constant of the formation of alanine anhydride. According to the model combined using Eqs. (1) and (4), the values of k_{rac} and k_{pip} were determined by SIMFIT for both the reactions from L-alanine and from D-alanine at the concentration range 0.02–0.5 M. The rate constants were basically independent on the concentration of alanine and on whether the racemization starts from L- or D-alanine. Thus, the rate constants k_{rac} and k_{pip} were ultimately determined to obtain a best fit by SIMFIT using all the reaction curves simultaneously (Table 1). In addition, the values of k_{rac} were less dependent on pH at 225–275°C, which is also in coincidence with previous studies [28,30].

In order to evaluate the rate of the formation of alanine anhydride, the disappearance of L-alanyl-L-alanine and that of the mixture of D,L-alanyl-D,L-alanine enantiomers to form alanine anhydride were monitored using the present system in a short time scale (Figs. 5 and 6) and the first-order rate plots were determined (Fig. 7). HPLC charts show the cyclization of L-alanyl-L-alanine within 5 s, in which the retention time of the product formed from L-alanyl-L-alanine coincided with that of authentic alanine anhydride (Fig. 5). During the cyclization of L-alanyl-L-alanine, the hydrolysis of L-alanyl-L-alanine was not observed, which is in contrary to a result observed for glycine [13]. Fig. 6 indicates that the cyclization of L-alanyl-L-alanine is much faster than the racemization of alanine and the formation of alanine anhydride from two alanine molecules. The cyclization from D,L-alanyl-D,L-alanine had similar rate to that from L-alanyl-L-alanine. Thus, the formation of alanine anhydride can

Table 1
The rate constants of racemization of alanine, formation of alanine anhydride, and cyclization of L-alanyl-L-alanine[a]

	T (°C)		
	225	250	275
pH = 7			
k_{rac} (s^{-1})	$(1.37 \pm 0.07) \times 10^{-3}$	$(3.69 \pm 0.10) \times 10^{-3}$	$(1.35 \pm 0.02) \times 10^{-2}$
k_{pip} ($s^{-1}\,M^{-1}$)	N.D.	N.D.	$(5.57 \pm 0.12) \times 10^{-2}$
k_{cyc} (s^{-1})	N.D.	N.D.	$(2.87 \pm 0.15) \times 10^{-1}$
pH = 4			
k_{rac} (s^{-1})	$(1.20 \pm 0.02) \times 10^{-3}$	$(3.52 \pm 0.27) \times 10^{-3}$	$(1.28 \pm 0.07) \times 10^{-2}$
pH = 10			
k_{rac} (s^{-1})	$(1.32 \pm 0.05) \times 10^{-3}$	$(2.82 \pm 0.03) \times 10^{-3}$	$(1.07 \pm 0.04) \times 10^{-2}$

[a] The rate constants were determined by SIMFIT using all the reaction curves in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.02–0.5 M alanine. N.D.: not determined.

be expressed by Eqs. (5.1) and (5.2).

$$\text{L-alanine} + \text{L-alanine} \xrightarrow[k_{dim}]{} \text{L-alanyl-L-alanine} \quad (5.1)$$

$$\text{L-alanyl-L-alanine} \xrightarrow[k_{cyc}]{} \text{alanine anhydride} \quad (5.2)$$

where k_{dim} is the rate constant of the formation of dipeptide Eq. (5.1) and k_{cyc} is that of cyclization of

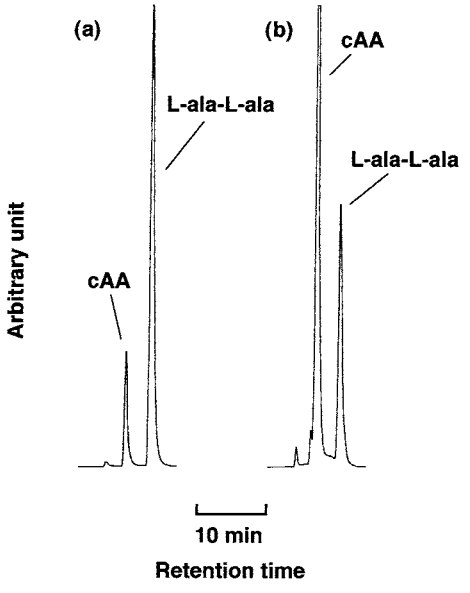

Fig. 5. HPLC chromatograms during cyclization of L-alanyl-L-alanine. The reactions were carried out in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.025 M L-alanyl-L-alanine at initial pH = 7.0 at 275°C. Residence time: (a) 0.40 s, (b) 4.03 s. Abbreviations: L-ala-L-ala, L-alanyl-L-alanine; cAA, alanine anhydride.

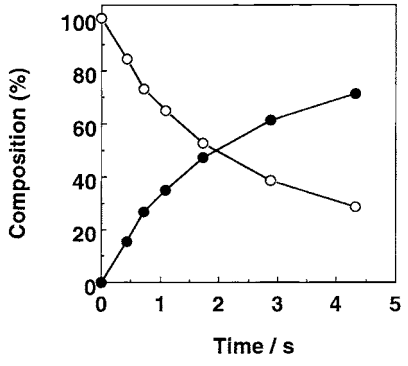

Fig. 6. The reaction curves for the formation of alanine anhydride from L-alanyl-L-alanine. The reaction was carried out in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.025 M L-alanyl-L-alanine at initial pH = 7.0 at 275°C. (○) L-alanyl-L-alanine; (●) alanine anhydride.

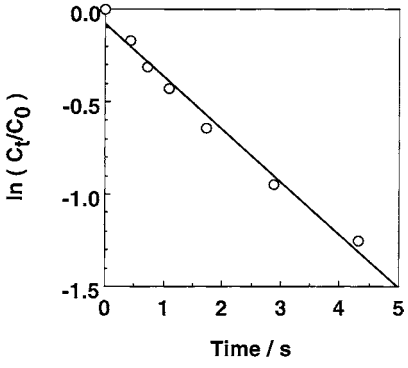

Fig. 7. The first-order rate plots of the formation of alanine anhydride from L-alanyl-L-alanine. The reaction conditions are the same as shown in Fig. 5.

linear L-alanyl-L-alanine. The magnitude of k_{cyc} determined from the slope in Fig. 7 is $0.287 \pm 0.015\ s^{-1}$ at 275°C, so the rate of cyclization is remarkably faster than that of the formation of alanine anhydride from two alanine molecules ($k_{pip} = 5.57 \times 10^{-3}\ M^{-1}\ s^{-1}$, Eq. (4)). The reaction curves shown in Figs. 3 and 6 demonstrate that the rate of formation of alanine anhydride and that of cyclization of L-alanyl-L-alanine are notably different. Thus, the magnitude of k_{dim} is considered to be approximately equal to k_{pip} since the formation of dimer is regarded as the rate determining step. In other words, the present system enabled to determine the rate constant of dimerization of alanine at 275°C. Further, the fact that no L-alanyl-L-alanine was detected during the formation of alanine anhydride supports this model. That is to say, L-alanyl-L-alanine does not remain as a primer for elongation since the cyclization of alanylalanine immediately occurs with much faster rate than the elongation of alanine. This is consistent with a previous study, in which the yield of glycine peptides was fairly low compared with glycine anhydride in the model reaction of a hydrothermal vent in deep ocean [7].

The racemization of leucine and phenylalanine was also followed using the chiral separation HPLC. The fact that by-products <10% were detected during the racemization of leucine and phenylalanine at 275°C and at 50–100 s may indicates that the cyclization occurred. First-order rate plots were determined on the basis of Eq. (3) as shown in Fig. 8, which gave good fits, and the rate constants are summarized in Table 2.

The apparent activation energy ($E_{a,app}$) was determined from the slopes of the Arrhenius plots of k_{rac} (Fig. 9, Table 3), and $E_{a,app}$ for alanine, leucine, and phenylalanine was in good agreement with a previous study carried out at lower temperatures [34].

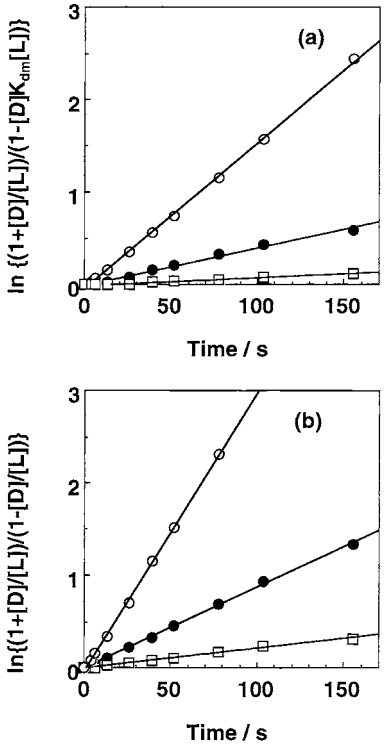

Fig. 8. The first-order rate plots for the racemization of L-leucine and L-phenylalanine. The reactions were carried out in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.01 M L-amino acid at initial pH = 7.0. (a) L-leucine, (b) L-phenylalanine. (○) 275°C; (●) 250°C; (□) 225°C.

3.2. Implications on the origin of life

Asymmetric biopolymers are considered to be essential to construct life on the earth. For instance, the information flow from DNA to protein in cell is warranted by the existence of asymmetric biopolymers. In other words, the biological functions of

Table 2
The rate constants of racemization of L-leucine and L-phenylalanine[a]

	T (°C)		
	225	250	275
L-leucine			
k_{rac} (s^{-1})	$(3.74 \pm 0.09) \times 10^{-4}$	$(1.97 \pm 0.03) \times 10^{-3}$	$(7.25 \pm 0.09) \times 10^{-3}$
L-phenylalanine			
k_{rac} (s^{-1})	$(1.04 \pm 0.01) \times 10^{-3}$	$(4.36 \pm 0.03) \times 10^{-4}$	$(1.42 \pm 0.02) \times 10^{-2}$

[a] The racemization was determined in the presence of 0.1 M NaCl, 0.05 M $MgCl_2$, 0.01 M amino acids.

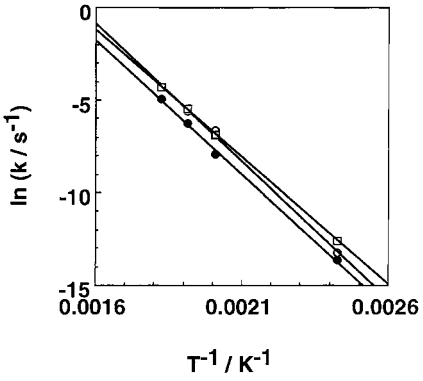

Fig. 9. Arrhenius plots for the racemization of alanine, leucine, and phenylalanine. Rate constants: 225–275°C (this study), 139°C [34].

Table 3
Apparent activation energy for the racemization of amino acids

Amino acids	$E_{a,app}$ (kJ mol^{-1})
Alanine	124
Leucine	120
Phenylalanine	115

proteins could have not appeared unless the rate of the primitive biological reactions was much greater than that of racemization of amino acids [22]. According to the present study, the half-lives of amino acids are estimated to be 32 day (alanine), 52 day (leucine), and 15 day (phenylalanine) at 110°C, so the racemization at 110°C is regarded as much slower than the enzymatic reactions even in hyperthermophiles. On the other hand, the half-lives of amino acids are 25–50 s at 275°C, 80–175 s at 250°C, and 250–950 s at 225°C, so the racemization of amino acids at 225–275°C might be considered as being too fast to construct primitive enzymatic reactions. However, on the contrary, if primitive enzymatic reactions were relatively so fast compared with the racemization of amino acids, the primitive enzymatic reactions could have played actual roles [19,25]. Thus, one should be careful when deciding whether the rate of racemization at hydrothermal conditions is too fast for prebiotic evolution or not.

On the other hand, it would be interesting to compare the stability of amino acid and nucleic acid monomers. The hydrolysis of 5′-ATP is much slower than the racemization of amino acids, in which the half-lives of 5′-ATP is 5.8 ms at 275°C, 21 ms at 250°C, and 82 ms at 225°C [25]. Thus, 5′-ATP is considered to be about 10^4 times more labile than the amino acids since racemization is the fastest degradation process for amino acids [13,14]. However, $E_{a,app}$ of 5′-ATP hydrolysis (120 kJ mol^{-1}) is not much different from that of the racemization of amino acids (115–124 kJ mol^{-1}), so the relative difference between the stability of 5′-ATP and amino acids are not much dependent on temperature. That is to say, the results support that amino acids are more stable than nucleic acids over wide range of temperatures.

Moreover, the fact that the rate of dimerization of alanine has similar magnitude to racemization seems to be a problem for the asymmetric peptide formation under hydrothermal conditions. Additionally, the present study shows that the hydrolyses of alanine anhydride and alanylalanine are much slower than the racemization of alanine. Besides, the fact that the cyclization of alanylalanine inhibits the oligomerization of alanine to form alanine peptides is analogous to that the cyclization of trinucleotides on montmorillonite inhibits the elongation of oligonucleotides [35]. These facts might suggest that higher yield of linear oligopeptides would occur using catalysts such as clay minerals [35,36], which could prohibit the cyclization of alanylalanine or catalyze the elongation of alanylalanine. Based on these considerations, it will be interesting to search possible conditions, where an asymmetric formation of oligopeptide could occur under hydrothermal conditions against racemization.

Acknowledgements

Engineering Laboratory and Workshop Center, Osaka Prefecture University facilitated by generously preparing a part of the flow system. We thank, Professors T. Nakahara and T. Yao in Osaka Prefecture University for the use of the HPLC apparatus and Professor G. von Kiedrowski in Ruhr-Universitaet Bochum for generously providing the computer program SIMFIT. This research was supported by the Sumitomo Foundation 1998, Japan.

References

[1] N.G. Holm (Ed.), Marine hydrothermal systems and the origin of life — special issue, Origins Life Evol. Biosphere 22 (1992) 5.

[2] S.L. Miller, J.L. Bada, Nature 334 (1988) 609.
[3] H. Yanagawa, F. Egami, Proc. Jpn. Acad. Ser. B 54 (1978) 331.
[4] H. Yanagawa, K. Kojima, J. Biochem. 97 (1985) 1521.
[5] W.L. Marshall, Geochim. Cosmochim. Acta 58 (1994) 2099.
[6] N.G. Holm, E.M. Anderson, Planet Space Sic. 43 (1995) 153.
[7] E. Imai, H. Honda, K. Hatori, A. Brack, K. Matsuno, Science 283 (1999) 831.
[8] N.R. Pace, Cell 65 (1991) 531.
[9] T. Oshima, Trans. Mater. Res. Soc. Jpn. 19B (1994) 1069.
[10] P. Forterre, C.R. Sciences de la vie/Life sciences, Acad. Sci. Paris 318 (1995) 415.
[11] P. Forterre, Cell 85 (1996) 789.
[12] R.H. White, Nature 310 (1984) 430.
[13] Y. Qian, M.H. Engel, S.A. Macko, S. Carpenter, J.W. Deming, Geochim. Cosmochim. Acta 57 (1993) 3281.
[14] H.C. Helgeson, J.P. Amend, Thermochim. Acta 245 (1994) 89.
[15] J. Bada, S.L. Miller, M. Zhao, Origins Life Evol. Biosphere 25 (1995) 111.
[16] R. Larralde, M.P. Robertson, S.L. Miller, Proc. Natl. Acad. Sci. USA 92 (1995) 8158.
[17] K. Kawamura, A. Yosida, O. Matumoto, Viva Origino 25 (1997) 177.
[18] M. Levy, S.L. Miller, Proc. Natl. Acad. Sci. USA 95 (1998) 7933.
[19] K. Kawamura, N. Kameyama, O. Matumoto, Viva Origino 27 (1999) 107.
[20] T.R. Cech, Proc. Natl. Acad. Sci. 83 (1986) 4360.
[21] G.F. Joyce, A.W. Shwartz, S. Miller, L.E. Orgel, Proc. Natl. Acad. Sci. USA 84 (1987) 4398.
[22] K. Kawamura, Viva Origino 28 (2000) 129.
[23] K. Kawamura, Nippon Kagaku Kaishi (1998) 255.
[24] K. Kawamura, Chem. Lett. (1999) 125.
[25] K. Kawamura, Bull. Chem. Soc. Jpn. 73 (2000) 1805.
[26] K.A. Kvenvolden, E. Peterson, Science 169 (1970) 1079.
[27] J.L. Bada, B.P. Luyendyk, J.B. Maynard, Science 170 (1970) 730–732.
[28] J.L. Bada, J. Am. Chem. Soc. 94 (1972) 1371.
[29] G.G. Smith, A. Khatib, G.S. Reddy, J. Am. Chem. Soc. 105 (1983) 293.
[30] R. Baum, G.G. Smith, J. Am. Chem. Soc. 108 (1986) 7325.
[31] G.G. Smith, R.C. Evans, R. Baum, J. Am. Chem. Soc. 108 (1986) 7327.
[32] A. Terfort, G. von Kiedrowski, Angew. Chem. Int. Ed. Engl. 31 (1992) 654.
[33] S. Steinberg, J.L. Bada, Science 213 (1981) 533.
[34] G.G. Smith, T. Sivakua, J. Org. Chem. 48 (1983) 627.
[35] K. Kawamura, J.P. Ferris, Origins Life Evol. Biosphere 29 (1999) 563–591.
[36] J.P. Ferris, A.R. Hill, J.R. Liu, L.E. Orgel, Nature 381 (1996) 59.