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Reaction of Amino Acids in a Supercritical Water-Flow Reactor Simulating Submarine Hydrothermal Systems

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A novel supercritical water flow-reactor was constructed in order to simulate submarine hydrothermal systems. The temperature of fluid inside the reaction tube could be monitored with thermocouples, which was proved to be different from the temperature outside the reaction tube. Oligomers of glycine up to tetraglycine were formed when a 100 mM glycine solution was heated at 200–350 °C for 2 minutes. None of glycine peptides were produced at 400 °C. It was suggested, however, that the formation of glycine condensates at higher temperature, including supercritical conditions of water. The stability of some amino acids under hydrothermal conditions was examined. ω-Amino acids and glutamic acid, which can form intramolecular condensates, showed higher stability than other α-amino acids at higher temperature, including supercritical conditions.

Amino acids together with nucleic acid bases and sugars are the building blocks for the present life system on earth. The formation processes of those components are among the prime questions about life. Oparin,[1] and then Urey,[2] proposed that the primitive atmosphere consisted of reducing materials. Their hypothesis was first examined by the historic experimental work of Miller,[3,4] who demonstrated that amino acids and many other organic compounds could be readily produced by spark discharges under highly reduced conditions. Other investigators showed that heat,[5,6] UV radiation,[7,8] shock waves[9] and laser-induced plasma energy[10] could be used to synthesize bioorganic compounds from a reducing starting mixture.

Rubey[11] challenged the Oparin–Urey model, which suggested that the early atmosphere was less reducing, but had carbon dioxide and carbon monoxide as major carbon compounds. Rubey's model was also supported by Levin,[12] Kasting,[13] and Matsui.[14] It has been reported that the formation of organic compounds from non or mildly-reducing atmospheres is more difficult than from highly reducing ones. Only cosmic rays could form bioorganic compounds from a very mildly reducing atmosphere effectively.[15]

In the late 1970's "local reducing" environments, called submarine hydrothermal systems (SHSs), were discovered.[16] SHSs are dynamic vent systems, and are thought to be "life reactors,"[17] which have non-equilibrium reaction systems.[18,19] These include gradients of the temperature-pressure, pH and concentration of various chemical components with the presence of many mineral catalysts, like montmorillonite clays, iron hydroxide oxide, manganates, sulfides and zeolites. Water that circulates through the systems transports dissolved substances from a high-temperature zone (350–400 °C) to an intermediate zone and then to a low-temperature zone (ca. 0–2 °C). It has been theoretically proposed that organic monomers, such as amino acids, are produced in the high-temperature zone of SHSs from H_2, CH_4, NH_3, H_2S, CO, HCN etc.[17] A number of both theoretical[20–25] and experimental[26–33] studies have been performed using closed systems (autoclaves) to simulate the SHSs.

Recently, several experiments have been performed to simulate reactions in SHSs by using flow reactors, since SHSs are not closed systems, as in autoclaves, but open flow systems. Imai et al.[19] examined the reactions of glycine in flow reaction systems. They reported peptide formation up to the glycine trimer at 200–250 °C without adding metal ions and control of the pH, where an electric heater was used and the reaction temperature was controlled solely by the temperature at a heater. Submarine hydrothermal systems might consist supercritical conditions at some points. Thus, it is needed to examine the reactions at and over supercritical conditions. Ikushima et al.[34] reported on the formation oligoglycines at supercritical conditions of water. They also used an electric heater; the reaction temperature was controlled in the same way as in the above-mentioned experiment. Preheaters were used in both cases mentioned above. Alargov et al.[35] also reported on the oligomerization of glycine, where the reaction temperature was set at 250–400 °C. In this experiment, water was also heated with an electric heater, and was then mixed with the reactants of room temperature. Although the temperature at the injection point was monitored, the actual temperature during the reactions was not monitored.

In order to examine the possible reactions of amino acids in SHSs, we constructed a novel supercritical water flow reactor (SCWFR). The SCWFR was equipped with an IR gold image furnace, and the temperature of the fluid inside the reaction tube could be monitored with thermocouples. In this study, we examined the polymerization of glycine and the stability of some amino acids at high temperatures, including supercritical conditions of water.

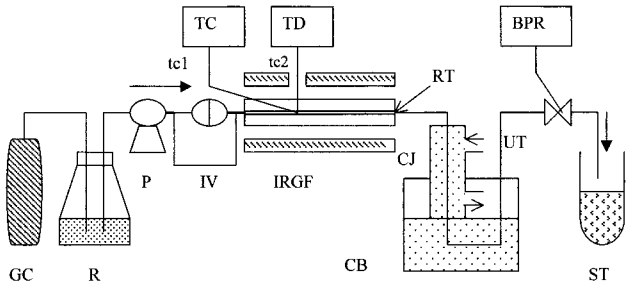


Fig. 1. Schematic Diagram of a Supercritical Water Flow Reactor (SCWFR). Abbreviations— GC: gas cylinder, R: reservoir, P: HPLC pump, IV: injection valve, IRGF: infrared gold image furnace, RT: reaction tube, TC: temperature controller, TD: temperature display, tc1 and tc2 thermocouples, CB: cold bath (0 °C), CJ: cooling jacket, UT: outlet tube, BPR: back pressure regulator, ST: sampling tube.

Experimental

Apparatus: A schematic diagram of SCWFR is shown in Fig. 1. A sample solution was pumped into the reaction tube with a HPLC pump (JASCO PU-1580). The fluid pressure was maintained at 25 MPa with a back-pressure regulator. The accuracy of the temperature controller (ULVAC/SINKU-RIKU, TPC-1000) was ±1.5 °C (±0.15%) bellow 200 °C, and improved to ±1 °C (±0.1%) at > 200 °C (up to 1700 °C). The fluid could be heated rapidly up to such a high temperature as 400 °C within a few seconds with an IR gold image furnace (ULVAC RHL-E 410 P) without preheating. The furnace was equipped with four 26.5 cm long quartz-tungsten lamps, which were situated inside a gold-plated body, which equally distributed heat to the reaction tube by radiation. The reaction tube (1.74 mm i.d., 3.3 mm o.d. × 43 cm) used was made of Hastelloy C-276, and connectors used in the high-temperature area of the system were made of Inconel (INC-200-3). The heating time could be altered by changing the flow rate: the heating time at a flow rate of 0.5 $dm^3\,min^{-1}$ was 2 min. Both the temperature outside and inside the reaction tube could be monitored with thermocouples. The thermocouple used to monitor the fluid temperature was sheathed with Inconel and was inserted into the reaction tube 15.5 cm from the inlet of the reaction tube. In the present experiments, the fluid temperature was monitored only at one point due to an equipment limitation.

Chemicals: Glycine, L-threonine, DL-serine and 2-oxohexamethyleneimine were purchased from Wako Pure Chemicals. DL-Aspartic acid, triglycine and tetraglycine were purchased from Sigma Chemicals. Diglycine and 6-aminohexanoic acid were from Nippon Rikagaku Yakuhin. Diketopiperazine, β-alanine, sarcosine, α-aminobutyric acid, pyroglutamic acid, DL-glutamic acid and γ-aminobutyric acid were purchased from Tokyo Kasei Kogyo. 5-aminovaleric acid, 2-piperidone and 2-pyrrolidinone were purchased from Aldrich Chemicals. All of the reagents used were of analytical grade. A Milli-Q Labo and a Millipore Simpli Lab-UV were used to eliminate organic compounds and metal ions from water. The solution in the reservoir was degassed by ultrasonic vibration under reduced pressure, and then bubbled with a gas mixture of nitrogen (99%) and hydrogen (1%) purchased from Takachiho Kagaku, pure hydrogen gas from Syowa Denko, or pure nitrogen gas from Taiyo Toyo Sanso.

Analytical Methods: An Ion-pair-HPLC system (pump: TOSOH DP 8020) was used to analyze peptides of glycine. The column used was an Inertsil ODS-80A (4.6 × 250 mm; GL Science), where an aqueous solution of 50 mM KH_2PO_4 and 7.2 mM $C_6H_{13}SO_3Na$ (pH 2.5) was used as a mobile phase in an isocratic elution mode. The flow rate of the mobile phase was 0.5 $dm^3\,min^{-1}$ (1 M = 1 $mol\,dm^{-3}$). A UV-detector (TOSOH UV-8020) was used, where the detection wavelength was 195 nm.

Amino acids were analyzed with a cation-exchange HPLC system (pumps: Shimadzu LC-6A), which was equipped with a Shim-pack ISC-07/S1504 (sodium type, 4.0 mm i.d. × 150 mm). The temperature of the column was maintained at 55 °C. Gradient elution was performed by using the following eluents: A) 0.07 M sodium citrate-perchloric acid (pH 3.20) with 7% ethanol, and B) 0.2 M sodium citrate–boric acid–NaOH (pH 10).[36] The flow rate of the carrier was 0.3 $dm^3\,min^{-1}$. Amino acids eluted from the column were detected with a Shimadzu RF-535 spectrofluorometric detector (excitation wavelength, 355 nm; emission wavelength, 435 nm) after post-column derivatization with *o*-phthalaldehyde and *N*-acetyl-L-cysteine. A sodium hypochlorite solution was used as the second derivatization reagent for the detection of imino acids, such as sarcosine.

Anhydrides of glutamic acid and ω-amino acids were analyzed with a reversed-phased-HPLC system (pump: TOSOH DP 8020). The column used was a Develosil RP AQUEOUS C 30-U (4.6 × 250 mm, Nomura Chemicals). The mobile phase used was a 25 mM KH_2PO_4–H_3PO_4 buffer (pH 3.0). The flow rate of the mobile phase was 1.0 $dm^3\,min^{-1}$. Eluents were monitored with a spectrophotometric detector (TOSOH UV-8020; wavelength, 205 nm).

Oligomerization of Glycine at Elevated Temperatures: We examined the possible formation of glycine oligomers and their alteration in SHSs. An aqueous solution of 100 mM glycine was pumped into the SCWFR at a flow rate of 0.5 $dm^3\,min^{-1}$ to heat at 200–400 °C under 25 MPa (over the critical pressure of water). The sample solution was quenched externally at 0 °C just after being heated. The resulting products were analyzed by cation-exchange HPLC and ion-pair HPLC.

Stability of Amino Acids at Elevated Temperatures: An aqueous solution of a mixture of ten amino acids (aspartic acid, threonine, serine, sarcosine, glutamic acid, α-aminobutyric acid, β-alanine, γ-aminobutyric acid, 5-aminovaleric acid and 6-aminohexanoic acid; 10 mM each) was heated at four different temperatures (250, 300, 350 and 400 °C) at 25 MPa for 2 min. An aliquot of each of the product solutions was hydrolyzed with 6 M HCl at 110 °C for 24 hours in a sealed tube. Products both before and after hydrolysis were analyzed by cation-exchange HPLC and by reversed-phase HPLC.

Results and Discussion

Temperature Profile of the Flow Reactor: Fig. 2 shows the temperature profile of the flow reactor, where the fluid temperature monitored inside the reaction tube is plotted against the temperature monitored on the surface of the reaction tube ("the set temperature"). The flow rate of the water eluent was 0.5 $dm^3\,min^{-1}$. In the ranges of 150–250 °C and 425–450 °C of the set temperature, the fluid temperature was higher than the set temperature; at 100 °C and in the range of 300–420 °C, on the contrary, the set temperature was higher than the fluid temperature. It seems to have been due to a change of the physicochemical properties.[37–50] The physical properties

Table 1. Effect of Pressure on the Supercritical Temperature of Water When Flow Rate Was 0.5 $dm^3\,min^{-1}$

Set temp/°C (Outside of the reaction tube)	Actual temperature inside the reaction tube/°C	
	At press. 25 MPa	At press. 21 MPa
406	374.5 °C (Supercritical fluid phase)	409 °C (Vapor/liquid phase)

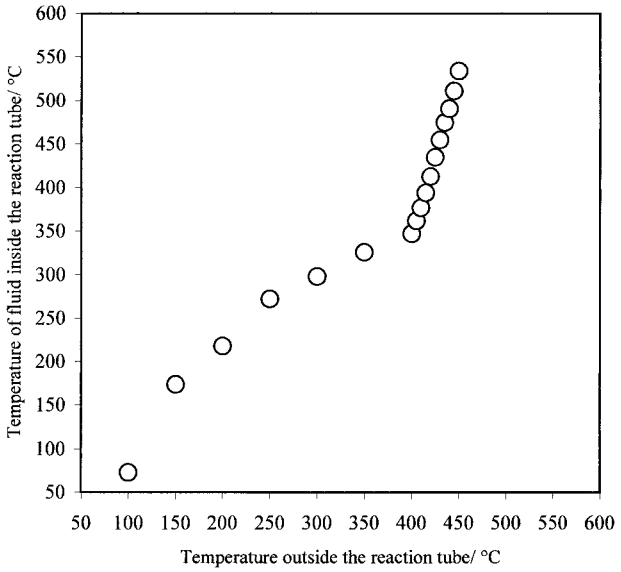


Fig. 2. Temperature of fluid inside the reaction tube vs set temperature (outside it). The flow rate of water eluent was 0.5 $dm^3\,min^{-1}$ under 25 MPa.

include thermal expansion, compressibility, heat capacity and viscosity of water for the variation of the temperature and pressure. For example, as the temperature rises from 25 °C to 300 °C, the density[41,42] of water decreases from 0.997 to 0.713 $g\,cm^{-3}$, its dielectric constant[43] decreases from 78.85 to 19.66 and its solubility parameter[44] decreases from 23.4 to 14.5 $(cal\,cm^{-3})^{1/2}$. Over the same temperature range, the ionic product[45] (dissociation constant) of water increases by three orders of magnitude, from $10^{13.99}$ to $10^{11.30}$. Changes of the above physical properties of water make water at 200 °C to have solvation properties more similar to methanol or nitrobenzene than those of water at room temperature,[46] and water at 300 °C shows solvation properties more similar to acetone.[47] These might be the reasons for the increase and decrease of the fluid temperature as well as causes for a fluid temperature higher than the set temperature. The temperature profile shown in Fig. 2 had an inflection point near to the supercritical point of water. The reason might be that the extent and strength of hydrogen bonding decreases peculiarly near to the critical point, and promotes the production of protons,[48] and also that the heat mass-transfer coefficient for horizontal flow becomes maximum at the supercritical point of water.[49] Thus, a change of even 1 °C from the set temperature caused drastic changes to the fluid temperature near the supercritical points of water. High-temperature and pressure conditions affect the chemical behavior of water.[50] Thus, it is necessary to monitor the fluid temperature to study reactions in water under supercritical or subcritical conditions.

Effect of Pressure on the Supercritical Temperature: The fluid temperature was monitored under two different pressures when the set temperature was 406 °C. As shown in Table 1, a big difference in the fluid temperature was monitored. When the pressure is lower than the critical pressure, water has the properties of vapor/liquid phases, and has a low dissociation constant (K_w) and a dielectric constant. When the pressure is over the critical pressure, it behaves like the supercritical phase, except that its dielectric constant and viscosity are much lower, because there are less hydrogen bonds among the water molecules.[51] The dissociation constant of water decreases as the density decreases.[52] The pressure effects the fluid temperature under the supercritical condition. It is thus necessary to monitor the fluid temperature, particularly in supercritical flow-reactor systems.

Table 2. The Effect of the Flow Rate on the Temperature in the SCWFR When the Set Temperature Was 300 °C

Flow rate/ $dm^3\,min^{-1}$	Fluid heating time/s	Temp. of fluid inside the reaction tube/°C
0.5	120	298
1	60	294
2	30	290
3	20	287
4	15	284
5	12	281
6	10	278
7	8.6	258
8	7.5	236
9	6.7	211
10	6	182

Pressure: 25 MPa.

Effect of the Flow Rate on the Fluid Temperature: Table 2 gives the variations in the temperature of the fluid inside the reaction tube when various flow rates were applied at the same set temperature (300 °C). At a lower flow rate of 0.5 $dm^3\,min^{-1}$, the fluid was heated for 120 seconds. When the flow rate was higher, the heating time became shorter. At a flow rate of 10 $dm^3\,min^{-1}$, for example, the fluid was heated only for 6 seconds, and the difference between the fluid temperature and the set temperature was increased. Thus, only the set temperature cannot give the actual temperature of the fluid inside the reaction tube. Evaluating Fig. 2, as well as Tables 1 and 2, it should be emphasized that monitoring the fluid temperature is essentially needed to obtain an accurate temperature of the reaction.

Oligomerization of Glycine in SCWFR: Figure 3 shows ion-pair chromatograms of the products when a glycine solution was heated at 350 °C and 400 °C. Diketopiperazine, diglycine, triglycine and tetraglycine were formed in the SCWFR. All of the four-glycine oligomers were detected in samples heated at 200–350 °C. The formation of tetraglycine at 350 °C was tentatively identified by the HPLC retention time, and due to the lower yield GC/MS was not performed.

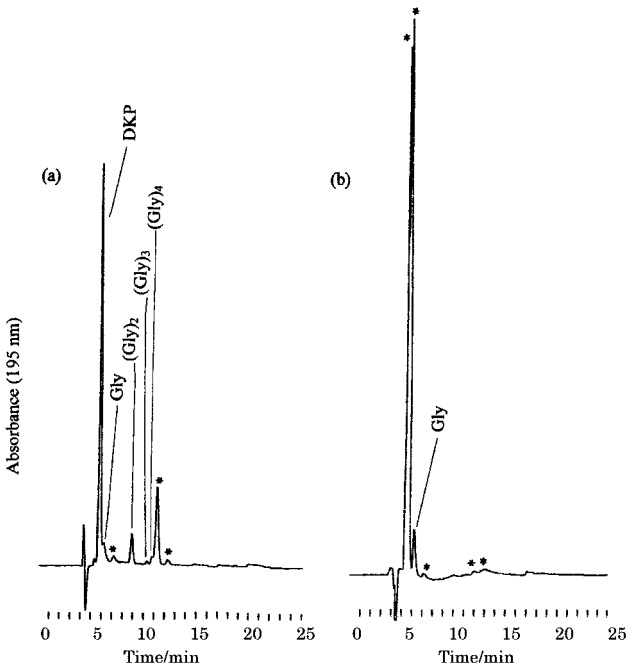


Fig. 3. Ion-pair chromatograms when 100 mM glycine solution was heated at (a) 350 °C and (b) 400 °C under 25 MPa. The flow rate of the mobile phase was 0.5 $dm^3\,min^{-1}$. Abbreviation— DKP: diketopiperazine. The unidentified peaks with asterisks were diminished after hydrolysis.

None of the four peptides were, however, found at 400 °C. It is suggested that the glycine reactions in a supercritical fluid are quite different from those in the liquid phase (at 200–350 °C). There were many other peaks of unknown compounds in all of the chromatograms of the unhydrolyzed products. Most of those peaks disappeared after acid hydrolysis.

Table 3 summarizes the yields of glycine and its oligomers. The yield of diketopiperazine was higher than any other oligoglycines, which implies that diketopiperazine is more stable than peptides of glycine. It also shows, at all temperatures, including 400 °C, that the glycine amount increased after acid-hydrolysis. It is suggested that condensates of glycine, which yielded amino acids after hydrolysis, formed even at such a high temperature as 400 °C.

Imai et al.[19] reported the results of glycine oligomerization, when 100 mM of a glycine aqueous solution was heated in a flow reactor at rather lower temperatures of 200–250 °C. They recycled the fluid, and the cycle time was 34 or 78 seconds according to the flow rate. Diketopiperazine, diglycine and triglycine were detected in the products without the addition of any catalysts. On the other hand, Ikushima et al.[34] reported on the formation of oligoglycines up to the trimer over supercritical conditions of water. Their heating time was as short as 0.2 to 1.1 seconds. The yields of glycine oligomers were higher in both of the experiments than our results. In both of the experiments, electric heaters were used and the reaction temperature was controlled by monitoring the temperature of the heater. Our present results suggested that it is difficult to obtain simple oligomers of glycine under supercritical conditions in actual submarine hydrothermal systems, where the heating time should be longer than minutes.

Stability of Amino Acids: Figure 4 shows the chromatograms of the hydrolyzed products when an aqueous mixture of 10 amino acids was heated at 400 °C for 2 minutes in the SCWFR. The reactants solution was bubbled with a mixture of nitrogen (99%) and hydrogen (1%) to make more reducing environments, like submarine hydrothermal systems.[27] Here, Fig. 4(a) was obtained by the injection of the product 50-times more than in the case of Fig. 4(b). Glycine and alanine obtained here were produced from other amino acids by thermal decomposition. Threonine and sarcosine were totally absent in the chromatograms because of their lower stability against heat.

Figure 5 shows the recovery ratio of amino acids after being heated in SCWFR before and after acid hydrolysis when the reactants solution was bubbled with pure (100%) nitrogen. The recovery ratios of amino acids after hydrolysis were much higher than those before hydrolysis, suggesting that the decrease in the concentration of amino acids was caused not only by decomposition, but also by the formation of condensates of amino acids that yielded amino acids after hydrolysis. Figures 6 and 7 represent the recovery ratios of amino acids when the reactant mixtures were bubbled with a mixture of nitrogen (99%) and hydrogen (1%), or pure hydrogen (100%), respectively. From Figs. 5(b), 6 and 7 it can be seen that the recovery ratio of amino acids was the highest when the reactants mixture was bubbled with a mixture of nitrogen (99%) and hydrogen (1%), and lowest when bubbled with pure nitrogen. Kohara et al.[27] reported on the stability of amino acids, where the reactants solution was heated in a closed system at 8 MPa and 200–350 °C; the recovery ratio was higher

Table 3. Products Obtained When 100 mM Glycine Aqueous Solution Was Heated at 200–400 °C under 25 MPa

Temp./°C	Products/mM						
	Before hydrolysis					After hydrolysis	
	Glycine (A)	DKP	Digly	Trigly	Tetragly	Glycine (B)	(B) − (A)
200	18.6	0.0178	0.0024	0.00014	trace	30.1	11.5
250	1.9	0.0177	0.0017	0.00007	trace	2.8	0.9
300	1.8	0.0082	0.0012	0.00009	trace	2.5	0.7
350	0.023	0.0057	0.00053	0.00004	trace	0.086	0.063
400	0.003	n.d.	n.d.	n.d.	n.d.	0.016	0.013

Abbreviation— DKP: diketopiperazine, digly: diglycine, trigly: triglycine, tetragly: tetraglycine. n.d.: not detected. Flow rate of the reactant: 0.5 $dm^3\,min^{-1}$.

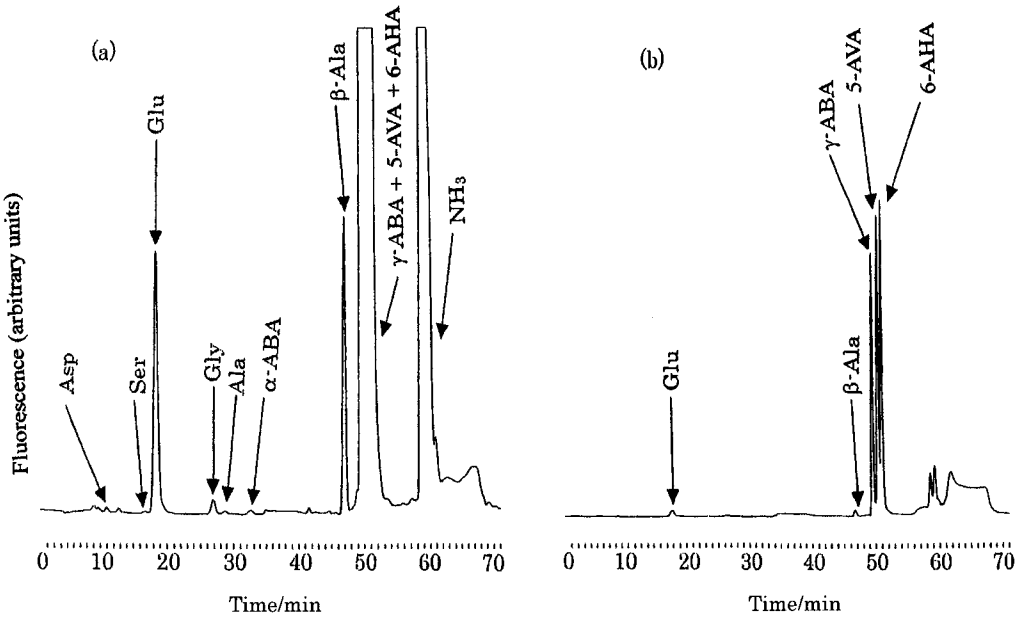


Fig. 4. Cation-exchange chromatograms of acid hydrolyzed products when an aqueous solution of ten amino acids was heated at 400 °C at the flow rate of 0.5 $dm^3\ min^{-1}$ under 25 MPa. Injection volume in (a) was 50 times more than that in (b). Abbreviations— Asp: aspartic acid, Thr: threonine, Ser: serine, Sar: sarcosine, Glu: glutamic acid, Gly: glycine, Ala: alanine, α-ABA: α-aminobutyric acid, β-Ala: β-alanine, γ-ABA: γ-aminobutyric acid, 5-AVA: 5-aminovaleric acid, 6-AHA: 6-aminohexanoic acid.

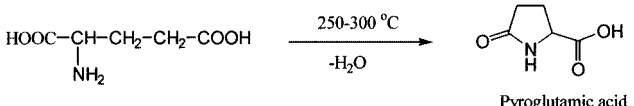
when a mixture of nitrogen (99%) and hydrogen (1%) was used as pressurized gas than when pure nitrogen was used. In both systems, the fugacity of hydrogen was proved to be important for the stability of amino acids. The difference in the recovery ratios was less in the present flow reactor system, which seems to be due to the lower partial pressure of hydrogen in the present system.

Shock et al.[52] theoretically discussed the stability of organic compounds in hydrothermal systems using the following equilibrium:

$$2CO_2 + 1/2N_2 + 9/2H_2 \rightleftharpoons \text{Glycine}(C_2H_5O_2N) + 2H_2O \quad (1)$$

Equation 1 suggests that the destruction of organic compounds, such as amino acids, was suppressed by the high fugacity of hydrogen, which also agrees with our results.

Aspartic acid, threonine, serine and sarcosine showed low stability against heat, compared to glutamic acid and the ω-amino acids. The recovery of hydrolyzed products of glutamic acid and ω-amino acids was much greater than the other α-amino acids, and their amounts drastically increased after acid hydrolysis. It seems to be due to the formation of intramolecular condensates of glutamic acid, γ-aminobutyric acid (γ-ABA), 5-aminovaleric acid (5-AVA) and 6-aminohexanoic acid (6-AHA) upon being heated (Eqs. 2–5) and the rings were opened after acid hydrolysis.



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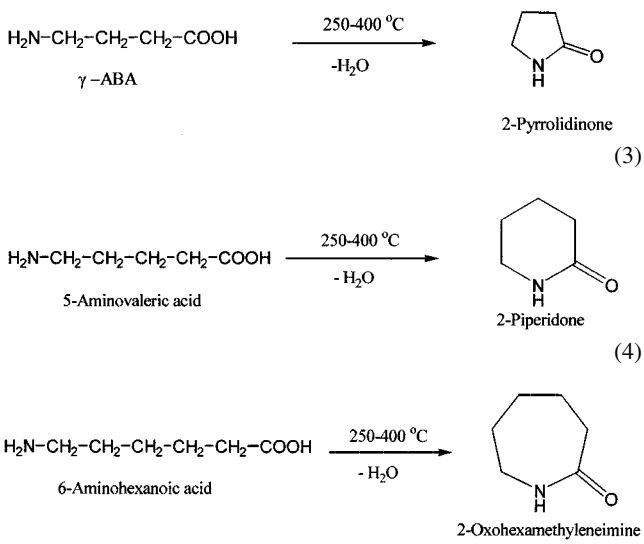

Figure 8 gives chromatograms of samples analyzed before and after hydrolysis by reversed-phase HPLC: (a) is that of authentic standards of pyroglutamic acid **1**; 2-pyrrolidinone **2**; 2-piperidone **3**; and 2-oxohexamethyleneimine **4**. (b) and (c) are the unhydrolyzed product by heating at 250 and 400 °C, respectively. It shows the formation of pyroglutamic acid, 2-pyrrolidinone, 2-piperidone and 2-oxohexamethyleneimine. All of the anhydrides were absent in the hydrolyzed products, as shown in Figs. 8(d) and (e), which suggests that the pyroglutamic acid, 2-pyrrolidinone, 2-piperidone and 2-oxohexamethyleneimine were hydrolyzed to amino acids. Pyroglutamic acid decreased when heated at higher temperature. Actually, as shown in Figs. 5(b), 6 and 7, that the recovery of glutamic acid was around 95% at 250–300 °C, while it was less than 5%

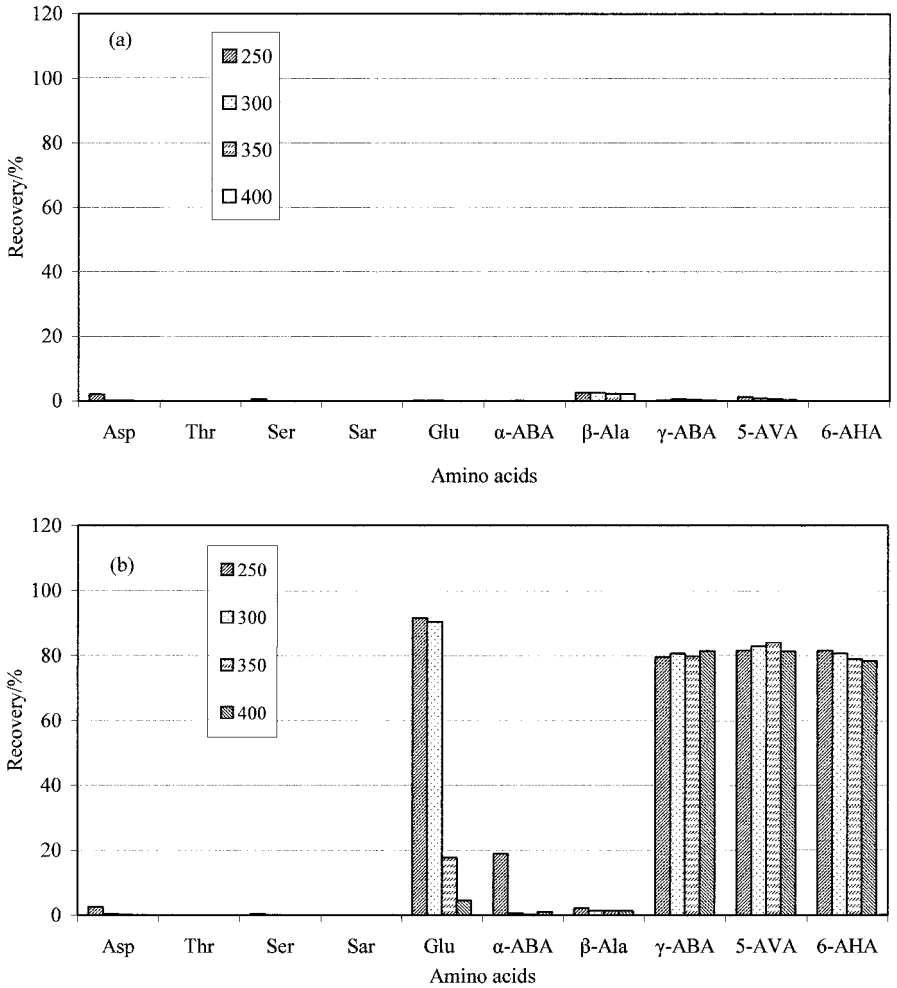

Fig. 5. Recovery ratios of amino acids when reactants solution was bubbled with pure nitrogen (100%). The flow rate of reactant solution was 0.5 $dm^3\ min^{-1}$ under 25 MPa. (a) Before hydrolysis, (b) After hydrolysis. Abbreviations— are shown in the caption of Fig. 4.

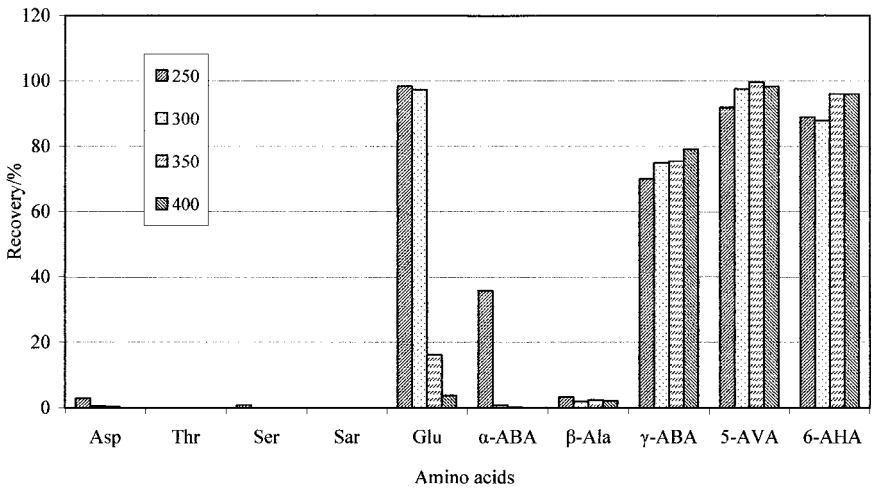

Fig. 6. Recovery ratios of amino acids (after hydrolysis), when reactants solution was bubbled with a mixture of nitrogen (99%) and hydrogen (1%). The flow rate of reactant solution was 0.5 $dm^3\ min^{-1}$ under 25 MPa. Abbreviations— are shown in the caption of Fig. 4.

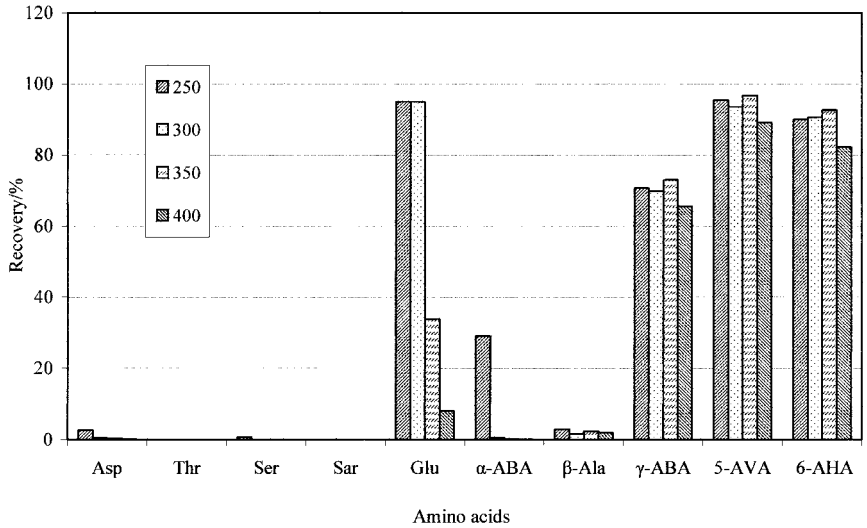

Fig. 7. Recovery ratios of amino acids (after hydrolysis) when reactants solution was bubbled with pure hydrogen (100%). The flow rate of reactant solution was 0.5 $dm^3\,min^{-1}$ under 25 MPa. Abbreviations— are shown in the caption of Fig. 4.

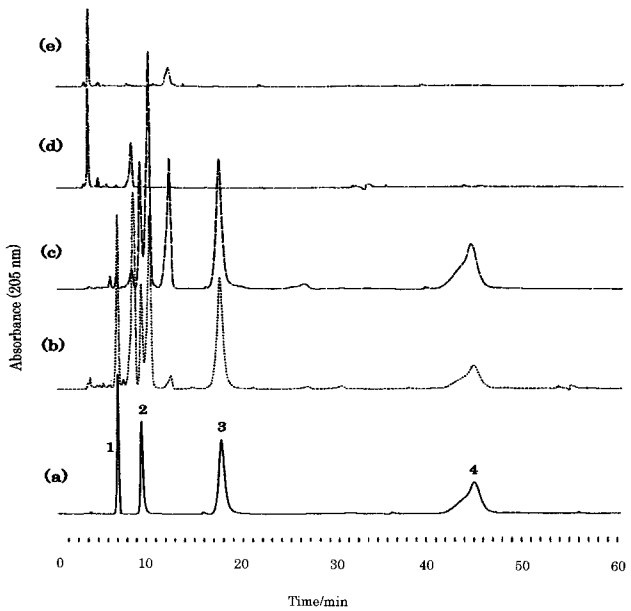

Fig. 8. Reversed-phase HPLC chromatograms of the products when an aqueous solution of ten amino acids were heated at the flow rate of 0.5 $dm^3\,min^{-1}$ under 25 MPa. (a) Standards: **1**. Pyroglutamic acid, **2**. 2-Pyrrolidinone, **3**. 2-Piperidone and **4**. 2-Oxomethyleneimine; (b) 250 °C, before hydrolysis; and (c) 400 °C, before hydrolysis; (d) 250 °C, after hydrolysis; and (e) 400 °C, after hydrolysis.

at 400 °C. The thermal stability of pyroglutamic acid is much lower than that of lactams of ω-amino acids, since pyroglutamic acid still has a carboxylic group, which is decomposed at a higher temperature (350 °C).

Islam et al.[53] reported on the formation of some ω-amino acids, such as β-alanine, γ-aminobutyric acid, 5-aminovaleric acid and 6-aminohexanoic acid, only at higher temperature (300–400 °C), when a mixture of KCN (0.1 M), HCHO (0.1 M, with 10–15% methanol) and NH_4HCO_3 (0.05 M) was heated. Hence, it is suggested that the formation of these ω-amino acids was possible at higher temperature, since the lactams of these amino acids are quite stable against heat. We can also say that the predominance of these ω-amino acids in some environments provides chemical markers of abiotic hydrothermal synthesis.

Conclusions

The present SCWFR was proven to have the following characteristics as a simulator of submarine hydrothermal systems: 1) Since an IR gold image furnace was applied to the present system, it could heat the fluid more rapidly without preheating than the previously reported reactors with electric furnaces. Thus, an alteration of the reactants before being heated up to the attempted temperature can be minimized.
2) The fluid temperature can be directly monitored with a thermocouple dipped into the fluid where the reactions are occurring. The monitoring of the fluid temperature was proved to be very essential for high-temperature and pressure experiments to obtain the actual reaction temperature. Monitoring of the fluid temperature was performed only at one point due to an equipment limitation. Thus, it remains one of our future perspectives to monitor the fluid temperature at multiple points to evaluate the temperature profile in detail.

Both of the characteristics described above are essential merits for the simulation of SHSs. It can therefore be used to find new types of reactions that possibly occurred during the course of chemical evolution in the primordial ocean.

Four types of oligoglycines, such as diketopiperazine, diglycine, triglycine and tetraglycine, were formed at 200–350 °C, together with many other unknown compounds, from a 100 mM glycine solution. The reaction of glycine in the supercritical water was different from that in the sub-critical water.

The stability of glutamic acid was higher than other α-amino acids at 250–300 °C due to the formation of an anhydride ring (pyroglutamic acid). ω-Amino acids, such as γ-aminobutyric acid, 5-aminovaleric acid and 6-aminohexanoic acid, showed a much higher recovery than α-amino acids, even over supercritical conditions of water. The higher recoveries were

due to the formation of lactams. These ω-amino acids could be chemical markers of abiotic hydrothermal synthesis. It was also shown that the stability of organic compounds depends on the oxidizing-reducing conditions of the systems.

It is suggested that some types of chemical evolution might require high-temperature and high-pressure environments close to SHSs, it is important to study the chemical evolution pathways in hydrothermal systems by using the SCWFR. Furthermore, it is also important to use metal ions to simulate SHSs in order to see what actually happening in natural systems.[19] These days, several projects have been conducted, including the ''Archaean Park'' project,[54] to study actual submarine hydrothermal systems. It is of interest to compare the results of simulation experiments with those observed in actual submarine hydrothermal systems.

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