ASTROBIOLOGY
Volume 11, Number 5, 2011
© Mary Ann Liebert, Inc.
DOI: 10.1089/ast.2011.0620

Mineral-Enhanced Hydrothermal Oligopeptide Formation at the Second Time Scale

Kunio Kawamura,* Hitoshi Takeya, Takao Kushibe, and Yuka Koizumi

Abstract

Accumulation of biopolymers should have been an essential step for the emergence of life on primitive Earth. However, experimental simulations for submarine hydrothermal vent systems in which high-temperature water spouts through minerals within a short time scale have not been attempted. Here, we show that enhancement of hydrothermal oligopeptide elongation by naturally occurring minerals was successfully verified for the first time by using a mineral-mediated hydrothermal flow reactor system (MMHF). MMHF consists of a narrow tubular reactor packed with mineral particles, and the enhancement or inhibitory activities of 10 types of naturally occurring minerals were successfully evaluated for an elongation reaction from $(Ala)_4$ to $(Ala)_5$ and higher oligopeptides in the absence of condensation reagents. It was unexpected that calcite and dolomite facilitated the elongation from $(Ala)_4$ to $(Ala)_5$ and higher oligopeptides with 28% yield at pH 7, while tourmaline, galena, apatite, mica, sphalerite, quartz, chalcopyrite, and pyrite did not show enhancement activities. These facts suggest the importance of carbonate minerals for the accumulation of peptide in primitive Earth environments.
Key Words: Hydrothermal reaction—Oligopeptide—Mineral—Calcite—Carbonate—Second time scale. Astrobiology 11, 461–469.

1. Introduction

Submarine hydrothermal vent systems are considered as possible environments for the accumulation of biopolymers on primitive Earth (Corliss *et al.*, 1981; Baross and Hoffman, 1985; Holm, 1992; Forterre, 1996; Martin *et al.*, 2008), although the hydrothermal origin-of-life hypothesis is still disputed (Miller and Bada, 1988; Miller and Lazcano, 1995; Galtier *et al.*, 1999). Because attempted simulation reactions have normally been carried out without the use of any catalysts and condensation agents, yields of the formation of oligopeptides from amino acids or short oligopeptides have not been so high. (Yamanaka *et al.*, 1988; Qian *et al.*, 1993; Yamagata and Inomata, 1997; Imai *et al.*, 1999; Kawamura *et al.*, 2005, 2009; Kawamura and Shimahashi, 2008). These reactions sometimes proceed within the second time scale (Kawamura *et al.*, 2005; Kawamura and Shimahashi, 2008). On the other hand, successful studies on the roles of minerals for chemical evolution support the hypothesis that minerals could have played important roles for the accumulation of biopolymers in primitive Earth conditions (Bernal, 1951; Schopf, 1983; Cairns-Smith *et al.*, 1992; Ferris and Ertem, 1992; Kawamura and Ferris, 1994; Ferris *et al.*, 1996, Bujdák and Rode, 1997; Milner-White and Russell, 2010). Thus, it would be informative to test the relative catalytic merits, or otherwise, of various minerals with regard to the synthesis of polypeptides (Steinberg and Bada, 1981; Rode, 1999; Kawamura, 2000). However, instrumentation for heterogeneous hydrothermal reactions in the presence of minerals, where a high-temperature aqueous medium and minerals encounter within a millisecond to second time scale, has not yet been attempted; the short time window is essential for the real-time evaluation of hydrothermal reactions since the hydrothermal reactions are generally fast. In the present study, we developed a mineral-mediated hydrothermal micro flow reactor system (MMHF), which consists of a high-temperature tubular reactor packed with mineral particles. By using MMHF, the enhancement and inhibitory activities of naturally occurring minerals for the spontaneous elongation from L-alanyl-L-alanyl-L-alanyl-L-alanine ($(Ala)_4$) to higher oligopeptides (Kawamura *et al.*, 2005) have been examined under hydrothermal conditions within the second time scale.

2. Materials and Methods

2.1. Chemicals

Naturally occurring minerals were obtained and treated as follows. Calcite (1), pyrite, sphalerite, galena, aragonite, and

---

Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, Sakai, Osaka, Japan.
*Present address: Department of Human Environmental Studies, Hiroshima Shudo University, Hiroshima, Japan.

montmorillonite were purchased from Alfer Aesar (Johnson Matthey Company, Japan). Calcite (1), sphalerite, galena, and aragonite were crushed and sieved to obtain a fraction (150–250 μm), and pyrite was sieved to obtain a fraction (75–150 μm). Calcite (1) was also sieved to obtain a fraction (40–75 μm). Montmorillonite was used without further treatment. Calcite (2) and zeolite were a gift from NFK (Nitto Funka Kogyo K.K.) Co. Ltd. Japan, which had been sieved in advance with 70 mesh (*ca.* 212 μm), and these were used without further treatment. Calcite (3) (Musashino, Japan) and dolomite (Cebu, Philippines) were gifts from JFK mineral Co. Ltd. Japan, and these were sieved (150–250 and 75–150 μm, respectively). Mica (C-83) and mica (CS-060DC) were a gift from Yamaguchi Mica Co. Ltd. Japan, and these were sieved to obtain a fraction (150–250 μm). Chalcopyrite (Agenosu-sawa, Takasu, Akita Prefecture, Japan) was obtained from Self Creation Inc. Japan, and it was crushed and sieved (75–150 μm). Tourmaline (Brazil) and apatite (Brazil) were obtained from Chie Co. Ltd. Japan. Tourmaline, which was sieved in advance at 325 mesh (44 μm), was used without further treatment. Apatite was grained and sieved to obtain a fraction (150–250 μm). Quartz sand was purchased from Wako Chemical Co. Ltd. Japan and sieved to obtain a fraction (250 μm <). Alanine oligopeptides, which included L-alanyl-L-alanyl-L-alanyl-L-alanine ($(Ala)_4$), were purchased from Sigma and Bachem. All other reagents used were of analytical grade.

The chemical formula of the minerals is as follows; the naturally occurring minerals frequently involve minor components: apatite, $Ca_5(PO_4)_3(F,Cl,OH)$; aragonite, $CaCO_3$; calcite, $CaCO_3$; chalcopyrite, $CuFeS_2$; dolomite, $CaMg(CO_3)_2$; galena, PbS; mica (muscovite), $KAl_2(Si_3Al)O_{10}(OH,F)_2$; montmorillonite, $(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$; pyrite, FeS_2; quartz, SiO_2; sphalerite, ZnS; tourmaline, $Na(Mg,Fe,Mn,Li,Al)_3Al_6(BO_3)_3Si_6O_{18}(OH,F)_4$; zeolite (mordenite), $(Ca,Na_2,K_2)Al_2Si_{10}O_{24} \cdot 7H_2O$. These minerals are normally considered to form under moderate- to high-temperature hydrothermal conditions (Bunno and Aoki, 1996).

2.2. Mineral-mediated hydrothermal flow reactor

The mineral-mediated hydrothermal flow reactor (MMHF) was designed on the basis of our previous method (Kawamura, 2000) and was attached with a high-temperature tubular reactor packed with naturally occurring mineral particles (Fig. 1). MMFR consists of a water reservoir, a high-pressure pump (Shimadzu LC-10AD), a sample injector (Rheodyne 7725i), a high-temperature tubular reactor [ϕ 1.5 mm × 150 mm (effective length 100 mm)] packed with natural mineral particles (MMHF), a temperature controller, a

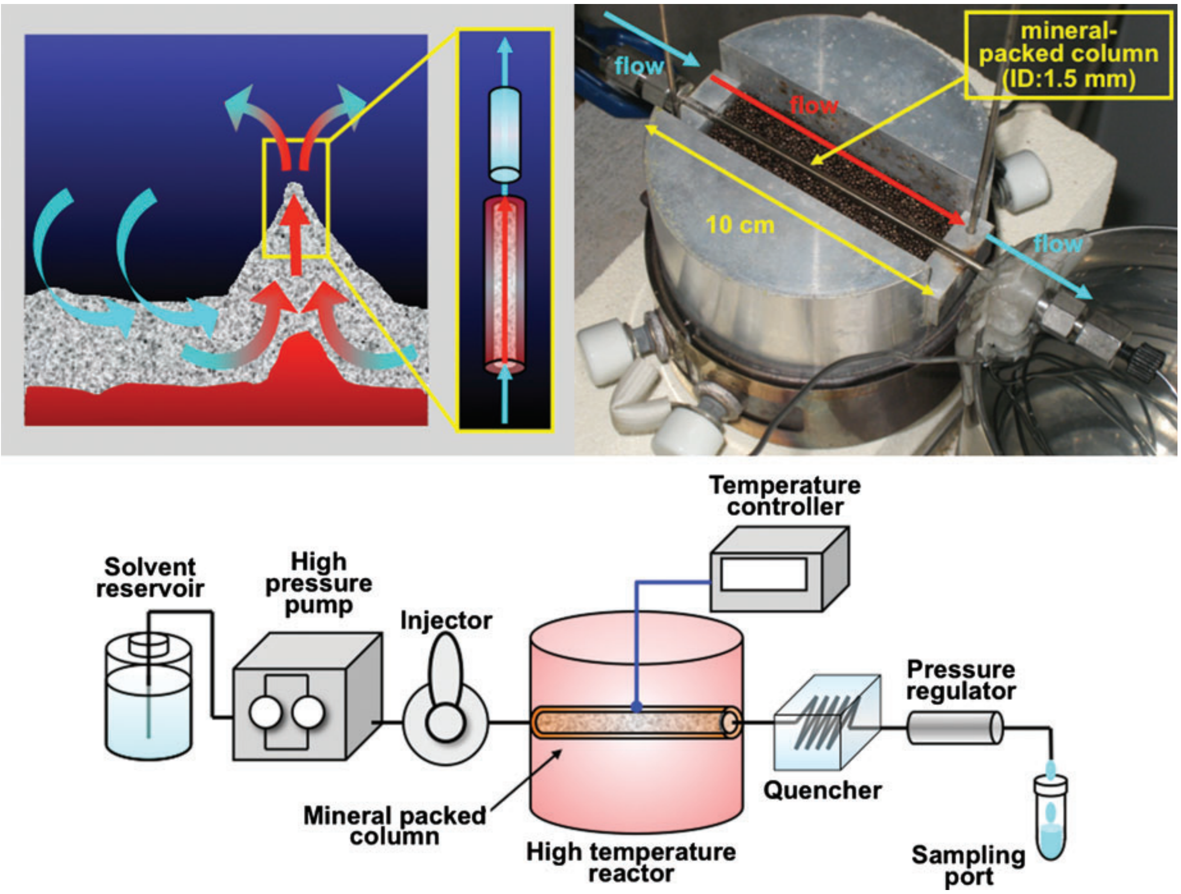

FIG. 1. Mineral-mediated hydrothermal flow reactor system (MMHF). Top left: the simplified pathway of aqueous solution in the hydrothermal vent system in deep sea. Top right: inside of the hydrothermal flow reactor, where the narrow column is packed with mineral particles. Bottom: the flow reactor system designed for hydrothermal reaction analysis with mineral-mediated high-temperature reactor. Color images available online at www.liebertonline.com/ast

block heater for the high-temperature tubular reactor, a quenching bath, a pressure regulator (Upchurch Scientific, P-880), and a sampling port. The thermocouple was located such that it connects to the high-temperature tubular reactor. Regulation of the flow rate controls the residence time during which the sample is exposed to high temperatures in the presence of mineral particles. It was established that the time required for heating up the sample solution to a target temperature is sufficiently short to the residence time where the sample solution is exposed at high temperature. It was confirmed that the quenching of the sample is also sufficiently fast (Kawamura, 2000). Filters were placed at both the inflow and outflow ends of the high-temperature tubular reactor to avoid entrainment and loss of the mineral particles. Both ends of the reactor were placed outside the block heater to avoid the thermal degradation of filtering materials. Different types of filters, such as a stainless-steel filter, a Teflon filter, and a cellulose filter, were examined to establish whether these are suitable for this system. Minerals in powdered form were obtained from different sources and used without further treatment. Massive minerals were ground to powders and sieved. MMHF enables analysis of the hydrothermal products catalyzed by minerals over the short time period required to form oligopeptides within 3–210 s at temperatures up to 300°C. The temperature was controlled at ±1%, and the pressure in the reactor was regulated at 12 MPa.

2.3. Sample preparation and high-performance liquid chromatography analysis

Samples containing NaCl, $MgCl_2$, and $(Ala)_4$ were injected to the MMHF system. The elongation reaction of $(Ala)_4$ was carried out by using MMHF within 3–210 s at temperatures 225–275°C at 12 MPa. The reaction products were analyzed with high-performance liquid chromatography (HPLC). HPLC was performed by a LC10A system (Shimadzu, Japan) with a reversed-phase column [2.0 mm (inner diameter) × 10 cm (length), CAPCELLPAK C18 UG120, Shiseido] with a guard column [2.0 mm (inner diameter) × 3.5 cm (length), CAPCELLPAK C18 UG120, Shiseido] with a gradient of 5 m*M* NaH_2PO_4 and 3.6 m*M* $CH_3(CH_2)_5SO_3Na$ at pH 2.65 (Eluent A) mixed with 10 m*M* NaH_2PO_4 and 7.2 m*M* $CH_3(CH_2)_5SO_3Na$ in 50% at pH 2.5 (Eluent B). A linear gradient of B% from 5% (0 min), 40% (15 min), 40% (25 min) was used for the analysis at the flow rate 0.2 mL/min at 35°C. All the detection was performed at 220 nm on the basis of the sensitivity and the low background.

Simulations of the reactions were carried out with Mathematica 5.2.

3. Results

3.1. Detection of mineral enhancement for oligopeptide elongation

The previous study regarding the reaction behavior of $(Ala)_4$ at high temperatures demonstrated that the reaction of $(Ala)_4$ at high temperatures forms mainly 3,6-dimethyldiketopiperazine (DKP) and alanine (Ala) but also results in the elongation to $(Ala)_5$ and higher oligopeptides during the degradation of $(Ala)_4$ (Kawamura *et al.,* 2005). In the present study, the influence of naturally occurring minerals from different sources was verified for the elongation of $(Ala)_4$. Montmorillonite, zeolite, aragonite, and mica (CS-060DC) were not applicable to MMHF measurements since the minerals blocked the flow in the tubular reactor. However, the influence of quartz sand, tourmaline, apatite, calcite (1), calcite (2), calcite (3), dolomite, chalcopyrite, sphalerite, galena, and mica was successfully observed within 3–210 s at 275°C. Although the reaction in the presence of pyrite was observable at 225°C and 250°C, the pressure of MMHF notably rose at 270°C so that the measurement at 275°C was not possible, presumably because of the deformation of pyrite releasing H_2S.

The HPLC profile of the reaction products with minerals resembles that obtained without minerals, where the disappearance of $(Ala)_4$ to form DKP and Ala are the main reactions and the formation of $(Ala)_5$ is observed (Fig. 2). The

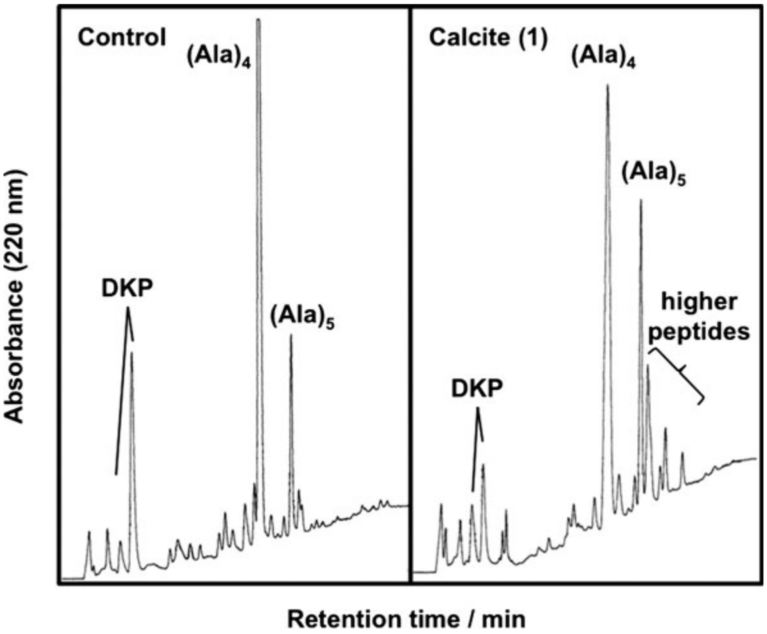

FIG. 2. HPLC analysis of the spontaneous elongation of $(Ala)_4$ to $(Ala)_5$ in the absence and presence of a calcite mineral. Left: control reaction. Right: in the presence of calcite (1) from Alfer Aesar (Johnson Matthey Company, Japan). Reaction conditions: $[(Ala)_4] = 1$ m*M*, [NaCl] = 0.1 *M*, $[MgCl_2] = 0.05$ *M*, pH = 7.0, 275°C, 12 MPa, 16.4 s.

HPLC peaks eluted after $(Ala)_5$ reflect the formation of $(Ala)_6$ and higher oligopeptides; the characterization of these products was extensively carried out in the previous study. The $(Ala)_5$ was identified by using an authentic $(Ala)_5$, and higher oligopeptides, including $(Ala)_6$, were characterized with MALDI-MS analysis (Kawamura *et al.*, 2005). In these reactions, partial racemization to form diastereomers occurs during the formation of $(Ala)_5$. The formation of two peaks of DKP is due to the racemization during the $(Ala)_4$ degradation. However, the peak for $(Ala)_4$ in HPLC does not show the difference of retention time among $(Ala)_4$ diastereomer. The HPLC profile of the reaction products of $(Ala)_4$ with L-Ala or D-Ala did not reveal any contrast between the products. This fact suggests that the peak of $(Ala)_5$ in HPLC includes $(Ala)_5$ diastereomers. The maximum extent of $(Ala)_5$ was determined from the $(Ala)_5$ formation curves (Fig. 3A). It is surprising that the formation of $(Ala)_5$ and higher oligopeptides from $(Ala)_4$ was notably enhanced in the presence of calcite (1), calcite (2), and dolomite. The yields of $(Ala)_5$ in the presence of these minerals reach to 13–14% within 15–30 s at pH 7 at 275°C. Some enhancement in the presence of calcite (3) was also observed. The reaction profile shows that the disappearance of $(Ala)_4$ and the formation of DKP are less dependent on the presence of minerals (Fig. 3B and 3C). The fact that there is no interval time in the reaction profiles supports the view that the temperature of the reaction samples immediately reaches the target temperature (Kawamura, 2000). On the other hand, the formation of higher oligopeptides proceeds simultaneously with that of $(Ala)_5$ (Fig. 3D). In the presence of calcite (1),

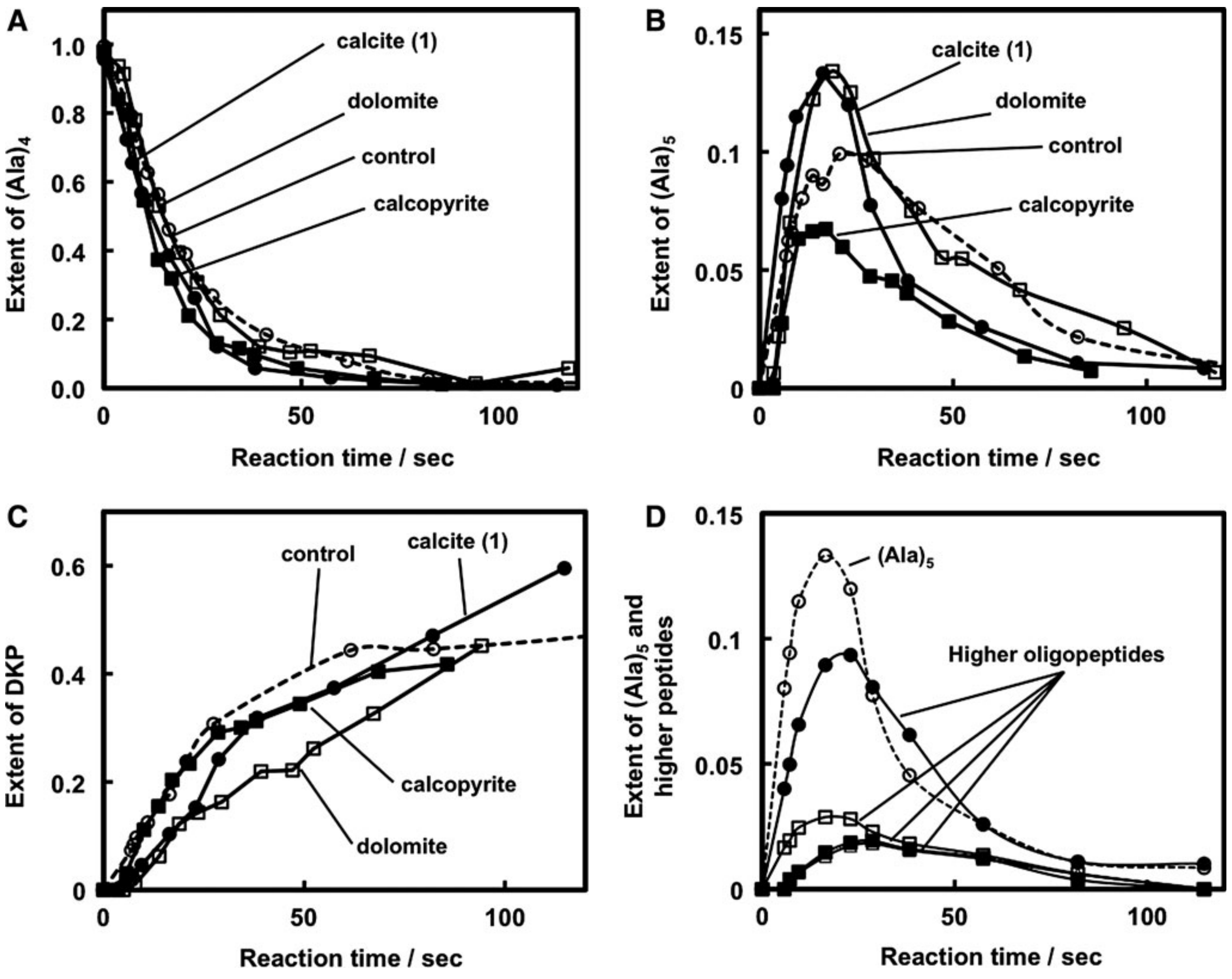

FIG. 3. Reaction curves of the formation of $(Ala)_5$ from $(Ala)_4$ in the presence of several minerals during the degradation of $(Ala)_4$ to 3,6-dimethyldiketopiperazine (DKP). (**A**) Formation of $(Ala)_5$. (**B**) The disappearance of $(Ala)_4$. (**C**) The formation of DKP. (**D**) Formation of $(Ala)_5$ and higher peptides using calcite (1). $(Ala)_5$ forms spontaneously and gradually degraded to DKP by the further incubation at extremely high temperature. Reaction conditions: 1 m*M* $(Ala)_4$, 0.1 *M* NaCl, 0.05 *M* $MgCl_2$ (initial pH = 7.0), at 275°C, at 12 MPa using MMHF. The samples were analyzed using a reversed-phase HPLC column. (A–C) Open circles, no mineral; closed circles, calcite (1); open squares, dolomite; closed squares, calcopyrite. (D) open circles, $(Ala)_5$; others, higher oligopeptides including $(Ala)_6$. The extent in vertical axis indicates the ratio of the products to the original $(Ala)_4$.

28% of the initial amount of $(Ala)_4$ was converted to $(Ala)_5$ and higher oligopeptides (Fig. 4). The influence of the particle size of calcite (1) was investigated, but no notable influence was detected.

Tourmaline, galena, apatite, mica (C-83), and sphalerite were less effective in the generation of $(Ala)_5$. Quartz sand and chalcopyrite showed weak inhibitory activities for the $(Ala)_5$ formations, where the maximum yield was reduced to 7% (Fig. 4). No enhancement of $(Ala)_4$ formation in the presence of pyrite could be detected at 225°C and 250°C.

3.2. Influence of pH

The pH value for the elongation of $(Ala)_4$ is an important factor since these minerals might change the pH conditions during the hydrothermal reactions. That is, the enhancement of elongation of $(Ala)_4$ might be due to an indirect effect of the pH change of aqueous solution by minerals. Thus, the contribution of pH for the elongation of $(Ala)_4$ was evaluated at different pH both in the absence and presence of minerals. The pH of sample solutions was adjusted at room temperature during the sample preparation. The pH values of samples shift to lower values at 275°C. In the absence of minerals, it was found that the yield of $(Ala)_5$ increases with increasing pH (Fig. 5A). Based on the reaction curves, the disappearance rate constants (k_{app}) of $(Ala)_4$ were calculated at pH 2–12 (Fig. 6), where the disappearance of $(Ala)_4$ follows the first-order kinetics.

On the other hand, the yields of $(Ala)_5$ and the disappearance rate of $(Ala)_4$ in the presence of calcite (2) were less dependent on pH (Fig. 5B). In addition, the pH of distilled water eluted through MMHF, which was controlled at 275°C, showed that the pH of the solution was 6.8.

4. Discussions

4.1. Reaction behavior

The total yields of $(Ala)_5$ and higher oligopeptides from $(Ala)_4$ in the presence of calcite (1), calcite (2), and dolomite without the use of a condensation agent are fairly high when compared to conventional hydrothermal formation pathways from amino acids or short peptides in the absence of minerals (Imai *et al.,* 1999; Kawamura *et al.,* 2005). Even calcite (3) showed some enhancement of activity as compared to other less-active minerals. According to our previous study, the elongation from $(Ala)_4$ to $(Ala)_5$ and higher oligopeptides in the absence of a mineral proceeds consecutively, so it is reasonable to assume that the elongation that occurs in the presence of minerals would be similar to elongation in the absence of minerals. However, the reaction curves of higher oligopeptides show that some of the higher oligopeptides form spontaneously with $(Ala)_5$ from $(Ala)_4$. This fact might indicate that some higher oligopeptides would form through different pathways rather than the simple elongation from $(Ala)_5$ to higher oligopeptides. Two different kinds of calcite and one dolomite showed notable enhancement for the $(Ala)_4$ elongations. This fact suggests that carbonate minerals would have facilitated the primitive oligopeptide formation on primitive Earth since carbonate minerals should have been formed under the high carbon dioxide pressure (Peter and Shanks, 1992), which is dependent on pH and temperatures (Macleod *et al.,* 1994).

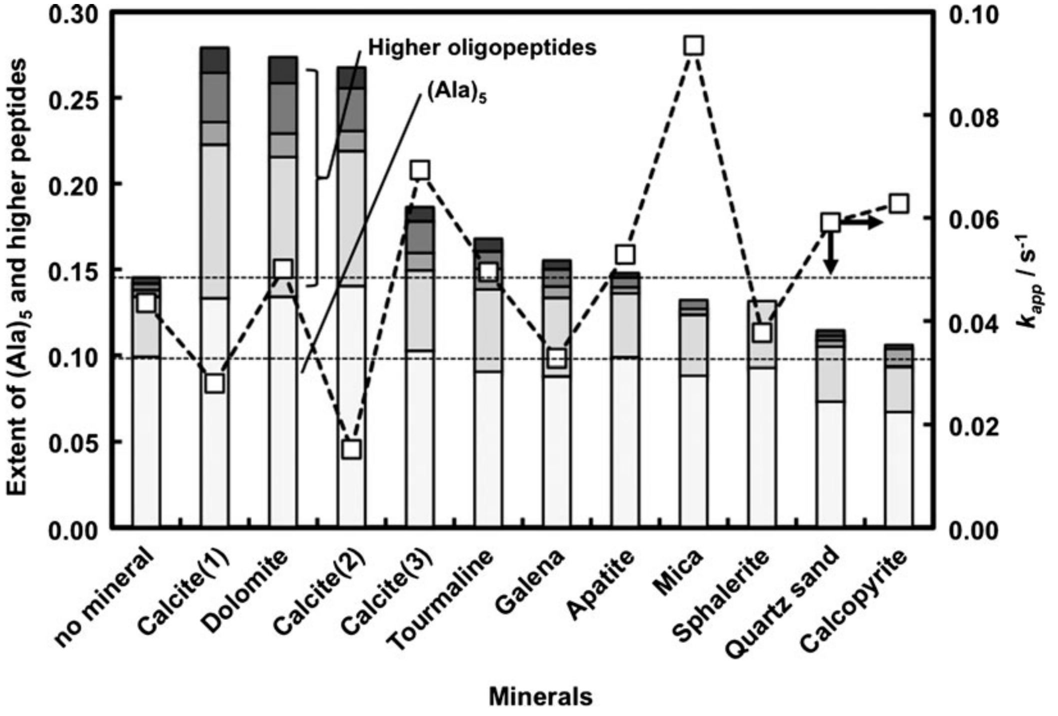

FIG. 4. Extent of $(Ala)_5$ and higher peptides at the maximum yield using different naturally occurring minerals. Bars: extent of $(Ala)_5$ and higher oligopeptides. Open squares: k_{app}. The extent in vertical axis indicates the ratio of the products to the original $(Ala)_4$.

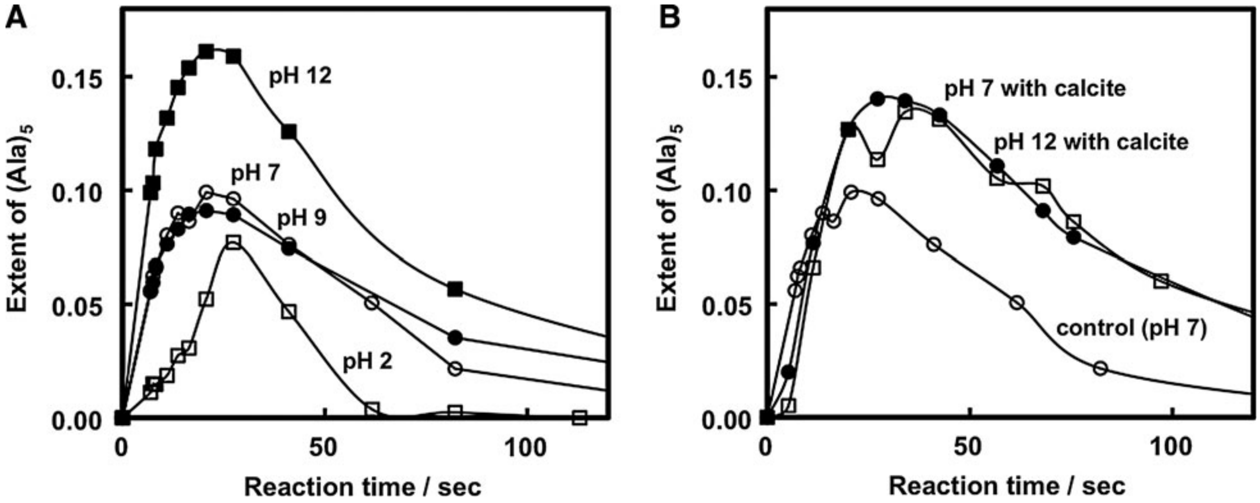

FIG. 5. The reaction curves for the appearance of $(\mathrm{Ala})_5$ from $(\mathrm{Ala})_4$ at different pH in the absence and presence of calcite mineral. Reaction conditions are the same as shown in Fig. 3. (**A**) No mineral: open circles, pH 7; closed circles, pH 9; open squares, pH 2; closed squares, pH 12. (**B**) Calcite (2): open circles, pH 7 without minerals; closed circles, pH 7 with calcite; open squares, pH 12 with calcite. The values of pH indicate the values at the sample preparation at room temperatures. The extent in vertical axis indicates the ratio of the products to the original $(\mathrm{Ala})_4$.

In contrast, silicate (quartz, mica, tourmaline) and phosphate (apatite) minerals showed a weak inhibition or no enhancement. In addition, it is noted that sulfide minerals (sphalerite, galena, and pyrite) did not show enhancement, and chalcopyrite showed a weak inhibitory activity. These facts are interesting in that, while further investigations for these types of mineral activities are needed, some investigators have predicted that sulfide minerals were an important factor in the chemical evolution of primitive biopolymer formation (Huber and Wächtershäuser, 1998; Huber *et al.*, 2003).

In the absence of minerals, the pH dependence for the disappearance rate of $(\mathrm{Ala})_4$ and the increase of $(\mathrm{Ala})_5$ suggests that the decrease of k_{cleavage} contributes the increase of $(\mathrm{Ala})_5$ (Figs. 5A and 6). Based on thermodynamic data, it is estimated that the pH of the present solution prepared at pH 7 and 12 at 25°C changes to around pH 4 and 9 at 275°C, where $(\mathrm{Ala})_4$ exist as zwitterions at pH 4 and anionic form at pH 9 at 275°C (Perrin, 1965; Helgeson, 1967; Sillen and Martell, 1971; Serjeant and Dempsey, 1979; Kawamura *et al.*, 2005). However, the k_{app} is not strongly dependent upon pH. This indicates that the distribution of cationic, zwitterionic, and anionic species of $(\mathrm{Ala})_4$ does not strongly affect the cleavage rate of $(\mathrm{Ala})_4$. On the contrary, the yield of $(\mathrm{Ala})_5$ in the presence of calcite (1) is not dependent on pH at 7 and 12. Furthermore, the pH of the distilled water, which is exposed in the high-temperature flow reactor at 275°C packed with calcite, was collected from the outlet of MMHF. The pH value of the distilled water showed that the pH value was 6.8. This indicates that calcite does not affect the pH value of solutions at high temperatures. This is consistent with the fact that calcite does not readily dissolve in water at high temperatures (Segnit *et al.*, 1962; Bischoff and Seyfried, 1978). The influence of calcite (1) for the elongation of $(\mathrm{Ala})_4$ conclusively demonstrates the net influence of this mineral.

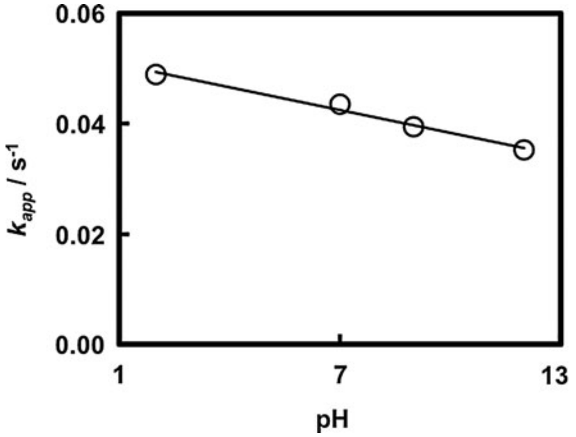

FIG. 6. The apparent rate constants for the disappearance of $(\mathrm{Ala})_4$ vs. pH.

4.2. Kinetic analysis

To evaluate the mechanism for the enhancement of $(\mathrm{Ala})_4$ elongations, kinetic simulations were carried out. Although the disappearance rate of $(\mathrm{Ala})_4$ and the extent of the formation of $(\mathrm{Ala})_5$ are dependent on the types of minerals present, the correlation between the disappearance rate and the yield of $(\mathrm{Ala})_5$ was not observed. The disappearance rates of $(\mathrm{Ala})_4$ follow the pseudo-first-order kinetics, and the reaction behavior in the presence of minerals is basically the same as that in the absence of minerals. Thus, the reaction mechanism proposed in the previous study (Kawamura *et al.*, 2005) is applied to the present mineral systems. Diastereomers formed by racemization cannot be distinguished on the basis of the present HPLC analysis. Thus, the disappearance rate of $(\mathrm{Ala})_4$ (k_{app}) is simply expressed by the combination of the cleavage rate of the peptide bonding of $(\mathrm{Ala})_4$ and the formation of $(\mathrm{Ala})_5$ from $(\mathrm{Ala})_4$ as shown in Eq. 1.

$$-\frac{d[(\mathrm{Ala})_4]}{dt} = k_{\mathrm{deg}}[(\mathrm{Ala})_4] + k_{\mathrm{elong}}[(\mathrm{Ala})_4][\mathrm{Ala}] \qquad (1)$$

The contribution of the degradation process of $(\mathrm{Ala})_4$ and the elongation of $(\mathrm{Ala})_4$ for the efficiency of $(\mathrm{Ala})_5$ formations was inspected by using a computer simulation on the basis of the following model.

$$(\text{Ala})_4 \overset{k_{\text{cleavage}}}{\rightarrow} (\text{Ala})_3 + \text{Ala or } 2(\text{Ala})_2 \qquad (2\text{-}1)$$

$$(\text{Ala})_5 \overset{k_{\text{cleavage}}}{\rightarrow} (\text{Ala})_4 + \text{Ala or } (\text{Ala})_3 + (\text{Ala})_2 \qquad (2\text{-}2)$$

$$(\text{Ala})_3 \overset{k_{\text{cyc3}}}{\rightarrow} \text{DKP} + \text{Ala} \qquad (2\text{-}3)$$

$$(\text{Ala})_2 \overset{k_{\text{cyc2}}}{\rightarrow} \text{DKP} \qquad (2\text{-}4)$$

$$(\text{Ala})_4 + (\text{Ala}) \overset{k_{\text{form}}}{\rightarrow} (\text{Ala})_5 \qquad (2\text{-}5)$$

Here, the degradation of $(\text{Ala})_4$ proceeds by the cleavage of peptide bonding of $(\text{Ala})_4$ to form Ala, L-alanyl-L-alanine ($(\text{Ala})_2$), and L-alanyl-L-alanyl-L-alanine ($(\text{Ala})_3$) as shown in Eq. 2-1. The cleavage process for $(\text{Ala})_5$ was also needed to define Eq. 2-2. The conversion from $(\text{Ala})_2$ and $(\text{Ala})_3$ to DKP immediately proceeds (Eqs. 2-3 and 2-4). On the other hand, the formation of $(\text{Ala})_5$ from $(\text{Ala})_4$ with Ala was expressed in Eq. 2-5. Based on this model, the influence of mineral for the apparent rate of disappearance of $(\text{Ala})_4$ shown in Eq. 1 is attributed to the cleavage shown in Eq. 2-1 or the formation shown in Eq. 2-5, or both.

Naturally, the extent of $(\text{Ala})_5$ increases with increasing magnitude of k_{form}. Besides, the extent of $(\text{Ala})_5$ increases with decreasing the magnitude of k_{cleavage} (Fig. 7). That is, the extent of $(\text{Ala})_5$ is determined on the basis of the relationship among the relative magnitudes of these rate constants. Where these minerals are absent, the yield of $(\text{Ala})_4$ increases with increasing pH, and the magnitude of k_{app} decreases with increasing pH. Thus, the enhancement of $(\text{Ala})_5$ yield in the absence of minerals would be due to the decrease of k_{cleavage}. On the contrary, based on the present data regarding the influence of minerals, there is no correlation between the disappearance rate constant of $(\text{Ala})_4$ (k_{app}) and the extent of $(\text{Ala})_5$ (Fig. 4). Thus, the apparent influence of minerals for the extent of $(\text{Ala})_5$ reflects the influence for the formation of $(\text{Ala})_5$ by the minerals regarding k_{form} rather than the stabilization of $(\text{Ala})_4$ by the minerals.

The reaction mechanism for the enhancement by calcite and dolomite is not clear at the present time. The fact that only calcite and dolomite show enhancement indicates that the enhancement would be due to the crystal framework of alkaline metal ion and carbonate. In addition, very recently, the catalytic hydrolysis of short oligopeptides by pyrite was detected (Marshall-Bowman *et al.,* 2010). This might be related with the inhibitory activity of sulfide minerals shown in this study. The trace amount of metal ions involved in the naturally occurring minerals might be important for the enhancement of $(\text{Ala})_5$ formation. In addition, the circulation time of deep-sea water through hydrothermal vent systems would be many orders of magnitude longer than the time scale used in the present study (Lalou *et al.,* 1993; Kadko and Butterfield, 1998; Jean-Baptiste *et al.,* 2004), something to be considered for future experimentation.

In conclusion, this study demonstrated that carbonate minerals facilitate the elongation of oligopeptides under hydrothermal conditions. This study also showed that the indirect influences of pH change and the stabilization of Ala in the presence of the minerals were not the reason for the enhancement of oligopeptide elongation. Furthermore, MMHF is a powerful tool for the kinetic investigations of the reaction behaviors in the presence of mineral particles under hydrothermal conditions. Naturally, the principle of MMHF would be applicable to a variety of fields regarding hydrothermal reactions with solid particles as well as chemical evolution of biopolymers.

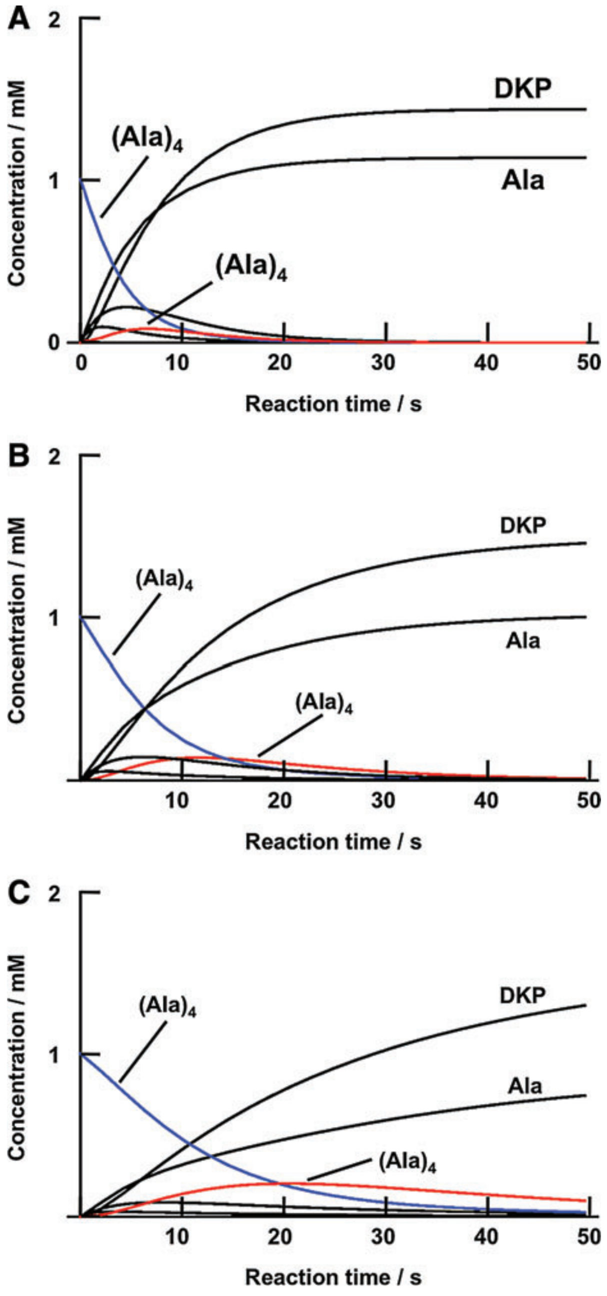

FIG. 7. Simulation of hydrothermal reaction of $(\text{Ala})_4$ system. Initial concentrations: $[(\text{Ala})_4] = 0.001\,M$, $[\text{Ala}] = [(\text{Ala})_2] = [(\text{Ala})_3] = [(\text{Ala})_5] = [\text{DKP}] = 0\,M$. Rate constants: k(DKP formation from $(\text{Ala})_2$) $= 0.25\,\text{s}^{-1}$, k(DKP formation from $(\text{Ala})_3$) $= 0.88\,\text{s}^{-1}$, k(form of $(\text{Ala})_5$ from $(\text{Ala})_4$) $= 150\,M^{-1}\,\text{s}^{-1}$. (**A**) k(cleavage of $(\text{Ala})_4$) $= 0.066\,\text{s}^{-1}$. (**B**) k(cleavage of $(\text{Ala})_4$) $= 0.033\,\text{s}^{-1}$. (**C**) k(cleavage of $(\text{Ala})_4$) $= 0.0165\,\text{s}^{-1}$. Color images available online at www.liebertonline.com/ast

Acknowledgments

We thank Nitto Funka Kogyo Co. Ltd. Japan, JFK mineral Co. Ltd. Japan, and Yamaguchi Mica Co. Ltd. Japan for the gift of minerals. This research was supported by Grant-in-Aid for Scientific Research in Japan, 20540476 from JSPS of Japan, a research grant from The Mazda Foundation, and Grant-in-Aid for Scientific Research 21200004 (2009-2011) on Innovative Areas from MEXT of Japan. The Engineering Laboratory and Workshop Center, Osaka Prefecture University, facilitated this work by generously preparing MMHF.

Abbreviations

Ala, alanine; DKP, dimethyldiketopiperazine; HPLC, high-performance liquid chromatography; MMHF, mineral-mediated hydrothermal flow reactor system.

References

Baross, J.A. and Hoffman, S.E. (1985) Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. *Orig Life Evol Biosph* 15:327–345.

Bernal, J.D. (1951) *The Physical Basis of Life,* Routledge and Kegan Paul, London.

Bischoff, J.L. and Seyfried, W.E. (1978) Hydrothermal chemistry of seawater from 25 degree to 350 degree C. *Am J Sci* 278:838–860.

Bujdák, J. and Rode, B.M. (1997) Glycine oligomerization on silica and alumina. *Reaction Kinetics and Catalysis Letters* 62:281–286.

Bunno, M. and Aoki, M. (1996) *Minerals and Rocks,* Hoikusha, Osaka.

Cairns-Smith, A.G., Hall, A.J., and Russell, M.J. (1992) Chapter 9 mineral theories of the origin of life and an iron sulfide example. *Orig Life Evol Biosph* 22:161–180.

Corliss, J.B., Baross, J.A., and Hoffman, S.E. (1981) An hypothesis concerning the relationship between submarine hot springs and the origin of life on Earth. *Oceanologica Acta* (Supplement) 4:59–69.

Ferris, J.P. and Ertem, G. (1992) Oligomerization of ribonucleotides on montmorillonite: reaction of the 5′-phosphorimidazolide of adenosine. *Science* 257:1387–1389.

Ferris, J.P., Hill, A.R., Jr., Liu, R., and Orgel, L.E. (1996) Synthesis of long prebiotic oligomers on mineral surfaces. *Nature* 381:59–61.

Forterre, P. (1996) A hot topic: the origin of hyperthermophiles. *Cell* 85:789–792.

Galtier, N., Tourasse, N., and Gouy, M. (1999) A non-hyperthermophilic common ancestor to extant life forms. *Science* 283:220–221.

Helgeson, H.C. (1967) Thermodynamics of complex dissociation in aqueous solution at elevated temperatures. *J Phys Chem* 71:3121–3136.

Holm, N.G., editor. (1992) Special issue—marine hydrothermal systems and the origin of life. *Orig Life Evol Biosph* 22:5–242 and therein.

Huber, C. and Wächtershäuser, G. (1998) Peptides by activation of amino acids with CO on (Ni,Fe)S surfaces: implications for the origin of life. *Science* 281:670–671.

Huber, C., Eisenreich, W., Hecht, S., and Wächtershäuser, G. (2003) A possible primordial peptide cycle. *Science* 301:938–940.

Imai, E., Honda, H., Hatori, K., Brack, A., and Matsuno, K. (1999) Elongation of oligopeptides in a simulated submarine hydrothermal system. *Science* 283:831–833.

Jean-Baptiste, P., Fourre, E., Metzl, N., Ternon, J.F., and Poisson, A. (2004) Red Sea deep water circulation and ventilation rate deduced from the He-3 and C-14 tracer fields. *J Mar Syst* 8:37–50.

Kadko, D. and Butterfield, D.A. (1998) The relationship of hydrothermal fluid composition and crustal residence time to maturity of vent fields on the Juan de Fuca Ridge. *Geochim Cosmochim Acta* 62:1521–1533.

Kawamura, K. (2000) Monitoring hydrothermal reactions on the millisecond time scale using a micro-tube flow reactor and kinetics of ATP hydrolysis for the RNA world hypothesis. *Bull Chem Soc Jpn* 73:1805–1811.

Kawamura, K. and Ferris, J.P. (1994) Kinetics and mechanistic analysis of dinucleotide and oligonucleotide formation from the 5′-phosphorimidazolide of adenosine on Na^+-montmorillonite. *J Am Chem Soc* 116:7564–7572.

Kawamura, K. and Shimahashi, M. (2008) One-step formation of oligopeptide-like molecules from Glu and Asp in hydrothermal environments. *Naturwissenschaften* 95:449–454.

Kawamura, K., Nishi, T., and Sakiyama, T. (2005) Consecutive elongation of alanine oligopeptides at the second time range under hydrothermal condition using a micro flow reactor system. *J Am Chem Soc* 127:522–523.

Kawamura, K., Takeya, H., and Kushibe, T. (2009) Effect of condensation agents and minerals for oligopeptide formation under mild and hydrothermal conditions in related to chemical evolution of proteins. *Adv Space Res* 44:267–275.

Lalou, C., Reys, J.-L., Brichet, E., Arnold, M., Thompson, G., Fouquet, Y., and Rona, P.A. (1993) New age data for Mid-Atlantic Ridge hydrothermal sites: TAG and Snakepit chronology revisited. *J Geophys Res* 98:9705–9713.

Macleod, M., McKeown, C., Hall, A.J., and Russell, M.J. (1994) Hydrothermal and oceanic pH conditions of possible relevance to the origin of life. *Orig Life Evol Biosph* 24:19–41.

Marshall-Bowman, K., Ohara, S., Sverjensky, D.A., Hazen, R.M., and Cleaves, H.J. (2010) Catalytic peptide hydrolysis by mineral surface: implications for prebiotic chemistry. *Geochim Cosmochim Acta* 74:5852–5861.

Martin, W., Baross, J., Kelley, D., and Russell, M.J. (2008) Hydrothermal vents and the origin of life. *Nat Rev Microbiol* 6:805–814.

Miller, S.L. and Bada, J.L. (1988) Submarine hot springs and the origin of life. *Nature* 334:609–611.

Miller, S.L. and Lazcano, A. (1995) The origin of life—did it occur at high temperatures? *J Mol Evol* 41:689–692.

Milner-White, E.J. and Russell, M.J. (2010) Polyphosphate-peptide synergy and the organic takeover at the emergence of life. *Journal of Cosmology* 10:3217–3229.

Perrin, D.D. (1965) *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London.

Peter, J.M. and Shanks, W.C., III. (1992) Sulfur, carbon, and oxygen isotope variations in submarine hydrothermal deposits of Guaymas Basin, Gulf of California, USA. *Geochim Cosmochim Acta* 56:2025–2040.

Qian, Y., Engel, M.H., Macko, S.A., Carpenter, S., and Deming, J.W. (1993) Kinetics of peptide hydrolysis and amino-acid decomposition at high-temperature. *Geochim Cosmochim Acta* 57:3281–3293.

Rode, B.M. (1999) Peptides and the origin of life. *Peptides* 20:773–786.

Schopf, J.W. (1983) *Earth's Earliest Biosphere, Its Origin, and Evolution*, Princeton University Press, Princeton.

Segnit, E.R., Holland, H.D., and Biscardi, C.J. (1962) The solubility of calcite in aqueous solutions—I The solubility of calcite in water between 75° and 200° at CO_2 pressures up to 60 atm. *Geochim Cosmochim Acta* 26:1301–1331.

Serjeant, E.P. and Dempsey, B. (1979) *Ionization Constants of Organic Acids in Aqueous Solution,* Pergamon, Oxford.

Sillen, L.G. and Martell, A.E. (1971) *Stability Constants of Metal-Ion Complexes,* The Chemical Society, London.

Steinberg, S.M. and Bada, J.L. (1981) Diketopiperazine formation during investigations of amino acid racemisation in dipeptides. *Science* 213:544–545.

Yamagata, Y. and Inomata, K. (1997) Condensation of glycylglycine to oligoglycines with trimetaphosphate in aqueous solution. II: Catalytic effect of magnesium ion. *Orig Life Evol Biosph* 27:339–344.

Yamanaka, J., Inomata, K., and Yamagata, Y. (1988) Condensation of oligoglycines with trimeta- and tetrametaphosphate in aqueous solutions. *Orig Life Evol Biosph* 18:165–178.

Address correspondence to:
Professor Kunio Kawamura
Department of Human Environmental Studies
Hiroshima Shudo University
Hiroshima Asaminami-ku Ozuka higashi 1-1-1 731-3195
Japan

E-mail: kawamura@shudo-u.ac.jp

Submitted 29 November 2010
Accepted 19 March 2011