Orig Life Evol Biosph (2017) 47:145–160
DOI 10.1007/s11084-016-9518-x

PREBIOTIC CHEMISTRY

Effects of Glycine, Water, Ammonia, and Ammonium Bicarbonate on the Oligomerization of Methionine

Rui Huang[1] · **Yoshihiro Furukawa**[1] · **Tsubasa Otake**[2] · **Takeshi Kakegawa**[1]

Received: 13 May 2016 / Accepted: 5 September 2016 /
Published online: 23 September 2016

Abstract The abiotic oligomerization of amino acids may have created primordial, protein-like biological catalysts on the early Earth. Previous studies have proposed and evaluated the potential of diagenesis for the amino acid oligomerization, simulating the formation of peptides that include glycine, alanine, and valine, separately. However, whether such conditions can promote the formation of peptides composed of multiple amino acids remains unclear. Furthermore, the chemistry of pore water in sediments should affect the oligomerization and degradation of amino acids and oligomers, but these effects have not been studied extensively. In this study, we investigated the effects of water, ammonia, ammonium bicarbonate, pH, and glycine on the oligomerization and degradation of methionine under high pressure (150 MPa) and high temperature conditions (175 °C) for 96 h. Methionine is more difficult to oligomerize than glycine and methionine dimer was formed in the incubation of dry powder of methionine. Methionine oligomers as long as trimers, as well as methionylglycine and glycylmethionine, were formed under every condition with these additional compounds. Among the compounds tested, the oligomerization reaction rate was accelerated by the presence of water and by an increase in pH. Ammonia also increased the oligomerization rate but consumed methionine by side reactions and resulted in the rapid degradation of methionine and its peptides. Similarly, glycine accelerated the oligomerization rate of methionine and the degradation of methionine, producing water, ammonia, and bicarbonate through its decomposition. With Gly, heterogeneous dimers (methionylglycine and glycylmethionine) were formed in greater amounts than with other additional compounds although smaller amount of these heterogeneous dimers were formed with other additional compounds. These results suggest that accelerated reaction rates induced by water and co-existing reactive compounds promote

✉ Yoshihiro Furukawa
furukawa@m.tohoku.ac.jp

[1] Department of Earth Science, Tohoku University, Sendai, Japan

[2] Division of Sustainable Resources Engineering, Faculty of Engineering, Hokkaido University, Sapporo, Japan

the oligomerization of less reactive amino acids during diagenesis and enhance the formation of peptides composed of multiple amino acids.

Keywords Prebiotic · Peptide · Pressure · Diagenesis · pH

Introduction

Proteins are essential components of modern life, playing key enzymatic roles in biological reactions. Proteins are primarily composed of polymerized amino acids. On prebiotic Earth, geological processes capable of inducing the abiotic polymerization of amino acids may have created primordial enzymes, thus contributing an essential ingredient for the origin of life. After Miller's experiment (Miller 1953), many researchers have reported the abiotic synthesis of amino acids from reduced atmospheres (Harada and Fox 1964; Ponnamperma et al. 1969). However, the most popular model currently postulates a slightly oxidized atmosphere on early Earth (Holland 1984; Kasting 1993). Thus, other processes for amino acid synthesis have been proposed, such as impact-induced formation and extraterrestrial delivery (Bernstein et al. 2002; Cronin and Moore 1971; Furukawa et al. 2015; Furukawa et al. 2009; Martins et al. 2013). As a result of such processes, several types of amino acids are presumed to have been dissolved in prebiotic oceans (Cleaves et al. 2009).

Several geological processes have been proposed for the creation of an environment suitable for the oligomerization of amino acids on early Earth. The tidal flat wet-and-dry cycle model is the traditional model for peptide formation. The effects of minerals, cations, and temperatures in this model have been investigated (Bujdák et al. 1995; Lahav et al. 1978; Rode 1999). A recent study demonstrated the formation of a 20-mer of glycine under an optimized temperature and wet-and-dry cycles (Rodriguez-Garcia et al. 2015). The submarine hydrothermal model is another well-known model for peptide formation (Cleaves et al. 2009; Imai et al. 1999; Kawamura et al. 2005; Lemke et al. 2009). The diagenesis model was recently proposed, in which amino acids are concentrated on the surface of clay minerals; compression and heating through diagenesis then dehydrate the sediments and convert the amino acids into peptides (Nakazawa 2008). Previous experimental studies of this model have focused on the effects of pressure and were conducted using pure, dry amino acid powders (Furukawa et al. 2012; Ohara et al. 2007; Otake et al. 2011). These studies demonstrated that high temperature and pressure conditions promote peptide formation. Natural sediments, however, contain minerals, water, ions, and other amino acids, as well as ammonia and bicarbonate, typical decomposition products of amino acids (Sato et al. 2004). Although the effects of several minerals on the oligomerization of amino acids have been investigated in the context of the tidal flat model (Bujdák et al. 1995; Bujdák and Rode 1999), the effects of water, ammonia, bicarbonate, and other amino acids on peptide formation remain unclear.

In previous investigations into the diagenesis model, the oligomerizations of Gly, Ala, and Val have been reported (Furukawa et al. 2012; Ohara et al. 2007; Otake et al. 2011). These amino acids have non-polar and relatively small side chains. Thus, they tend to oligomerize more easily than larger amino acids, whose sterically bulky side chains disrupt nucleophilic reactions necessary for the formation of a peptide bond. However, for the formation of protein-like catalytic molecules with specific configurations, the incorporation of more functional amino acids is essential. Methionine (Met) is a proteinogenic amino acid with a relatively large side chain that contains a sulfur atom which is contributing to the folding in proteins. Further,

Met is encoded by the start codon and is used to initiate biological protein synthesis in almost all living organisms. However, methionine's sterically bulky side chain have a potential to makes the abiotic formation of a peptide bond difficult. Indeed, a dimer is the longest peptide that has been formed in experimental simulations of prebiotic Met oligomerization (Li et al. 2008), much shorter than the glycine 6-mer produced in a similar experiment (Rode and Schwendinger 1990). In addition, the properties of Met and Met-based peptides at high temperatures and pressures are completely unknown. Thus, the oligomerization of Met was investigated in the present study under high temperature (175 °C) and high pressure (150 MPa) conditions. In addition to temperature and pressure effect, we investigated the effects of glycine, water, ammonia, ammonium bicarbonate, and pH on Met peptide formation and decomposition.

Experimental

Materials

L-methionine powder (>99 %), glycine powder (>99 %), ammonium bicarbonate, and sodium hydroxide (>95 %) from Wako Pure Chemical Industries, Ltd. and aqueous ammonia (28 %) from Sigma-Aldrich. Ammonium bicarbonate was used to investigate the effects from bicarbonate because it decomposes at approximately 60 °C and produces equal amounts of water, ammonia, and carbon dioxide. Ultrapure water was prepared from deionized water with Simplicity UV (Millipore), resulting in a final resistance of 18.2 MΩ.

For analysis of peptides and amino acids, acetonitrile (LC/MS grade; Wako Pure Chem.), undecafluorohexanoic acid (ion-pair reagent for LC/MS; TCI), and ammonium formate (>99.9 %; Sigma-Aldrich) were used as eluents. Most peptide standards were purchased from Bachem AG for identification and quantification with a high-performance liquid chromatograph connected to a tandem mass spectrometer (LC/MSMS).

Experimental Methods

For assessment of peptide formation and Met degradation, experiments were conducted by incubating 0.42 mmol of L-methionine under high temperature and pressure, either alone or with varying concentrations of additional compounds. Conditions tested included: (a) Met + 0.43 mmol H_2O, (b) Met + 4.3 mmol H_2O, (c) Met + 1.48×10^{-1} M aqueous ammonia ($NH_{3(aq)}$) in 0.43 mmol H_2O, (d) Met + 1.48×10^{1} M $NH_{3(aq)}$ in 0.43 mmol H_2O, (e) Met + 1.48×10^{-1} M ammonium bicarbonate solution ($NH_4HCO_{3(aq)}$) in 0.43 mmol H_2O, (f) Met + 1.48×10^{1} M $NH_4HCO_{3(aq)}$ in 0.43 mmol H_2O, (g) Met + NaOH in 0.43 mmol H_2O, adjusted to pH 13.7, or (h) a mixture of 0.21 mmol Met and 0.21 mmol Gly. The above starting materials were each incubated at 175 °C and 150 MPa; replicates were incubated for 3, 12, 24, 48, or 96 h.

In natural sediments, the temperature and pressure increase via a geothermal gradient. In this experiment, however, excess temperature was applied to the system to create a higher temperature-pressure relationship than that of the modern geothermal gradient (Hopkins et al. 2010). This is because excess heating accelerates reactions that normally occur on a geological timescale, enabling the evaluation of reactions on a laboratory timescale. The starting materials were sealed by arc welding in a gold (99.95 % purity, 0.2-mm thick) capsule with an external diameter of 5.5 mm and external length of 25 mm. The gold capsule was washed with a 10 %

HNO_3 solution and ultrapure water and then heated at 800 °C for 5 h to remove any organic contaminants. Although pore space in the capsule was diminished as much as possible, a small volume of air contamination (up to approximately 10 % of the volume of the starting materials) may have been present.

Gold capsules were placed in a test-tube autoclave system with water as the pressure medium. After raising the pressure to 150 ± 0.5 MPa, the temperature was raised to 175 ± 0.5 °C over the course of approximately 60 min. At the end of each experiment, heating was stopped and the experimental system was quenched to ambient temperature within 5 min. All capsules were confirmed to exhibit no weight change before and after the experiments, implying that they were perfectly sealed and no contamination occurred during the experiments. Capsules were stored at −20 °C after the experiments until further analysis. They were further cooled with liquid nitrogen just before opening. Capsules were dried in a vacuum for more than 12 h to remove any volatile compounds (e.g., H_2O, NH_3, CO_2, and CH_3SH) that were used as starting materials or produced during the experiments.

Supposing an ocean depth of 1000 m, a pressure of 150 MPa corresponds to ~6000 m beneath the ocean floor. This depth would correspond with a temperature of ~150 °C supposing a geothermal gradient of 30 °C/km and a sediment density of 3.0 g/cm^3. The temperature used in this experiment (175 °C) was higher than this expected temperature. Chemical reactions in deep marine sediments take several million years, and such long durations cannot be simulated in the laboratory. Therefore, it is necessary to accelerate the reaction speed. For this purpose, a higher temperature was used, as has been done in previous high temperature and pressure experiments.

Analytical Methods

Dried samples were analyzed with LC/MSMS (2695 Separation Module and Quattro micro API; Waters). For peptide analysis, a reverse-phase column (Atlantis T3, 3 μm, 2.1 × 130 mm; Waters) was used at 30 °C for separation. Two eluents were used with a total flow rate of 0.2 mL/min: eluents A (5 mM undecafluorohexanoic acid) and B (acetonitrile). The composition of the eluents was altered according to the following gradient program: A/B = 98/2 initial ratio, followed by 85/15 at 30–40 min, then 60/40 at 70 min. An aliquot of the products (~1 mg) was dissolved into 1 mL water containing 2 vol% acetonitrile. A portion of the sample solution (5 μL) was analyzed with the LC/MSMS system. A positive mode of electrospray ionization (ESI) was used to ionize the products. Capillary voltage, source temperature, and desolvation temperature were set at 3.5 kV, 110 °C, and 350 °C, respectively. Desolvation and cone gas flow rates were fixed at 600 and 50 L/h, respectively.

The peptides analyzed in this study were Met dimer (Met_2), Met trimer (Met_3), Met cyclic dimer (Met_{DKP}), glycine dimer (Gly_2), glycylmethionine (Gly-Met), and methionylglycine (Met-Gly). Peptides in the products were quantified based on their relative peak area on selected-ion resonance (SIR) chromatograms versus that of standard peptides. This analytical method has the ability to detect structural isomers separately with different retention times but cannot distinguish between stereoisomers.

For amino acid analysis, a hydrophilic interaction liquid chromatography column (ZORBAX HILIC Plus, 2.1 × 100 mm 3.5 μm; Agilent Technology) was used at 30 °C for separation. Two eluents were used for separation with a total flow rate of 0.2 mL/min: eluents A (10 mM ammonium formate, pH 3) and B (acetonitrile). The initial A/B ratio was 10/90, which changed to 30/70 at 20 min. ESI was used for ionization. Capillary voltage, source

temperature, and desolvation temperature were set at 3.5 kV, 110 °C, and 350 °C, respectively. Desolvation and cone gas flow rates were fixed at 600 and 50 L/h, respectively. An amino acids mixture of standard solution type H (Wako Pure Chem.) was used as the standard for identification and quantification. Quantification of amino acids was also conducted using relative peak area on SIR chromatograms, as in the peptide analysis.

The recovery of solid residues after drying of experimental products was calculated as follows:

$$\text{Recovery} = W_{dry}/W_{aa} \times 100\ (\text{wt}\%) \tag{1}$$

where W_{aa} and W_{dry} represent the weight of amino acids in the starting materials and total weight of dried products, respectively. The yields of produced peptides and recovered amino acids, Y_{pep} and Y_{aa}, were calculated as:

$$Y_{pep} = 100 \times C_{pep} \times W_{aa}/M_{aa}\ (\text{mol}\%) \tag{2}$$

$$Y_{aa} = 100 \times C_{aa} \times W_{aa}/M_{aa}\ (\text{mol}\%) \tag{3}$$

where M_{aa} and C_{pep} represent the molar amount of amino acids in the starting material and the concentration of each peptide in the dried solid residues (mol/mg), respectively.

Sulfur contents and sulfur isotope composition of the dried residues were measured using an elemental analyzer (Carlo Erba, EA1108) connected to an isotope ratio mass spectrometer (Finnigan Mat, MAT 252). The sulfur isotope composition of products is reported with the conventional δ notation ($\delta^{34}S_{CDT}$) as the per mil (‰) deviation of the $^{34}S/^{32}S$ ratio relative to that of Canyon Diablo Troilite (CDT) and expressed as the difference from the initial value as follows:

$$\Delta^{34}S = \delta^{34}S_{product} - \delta^{34}S_{initial} \tag{4}$$

Results

Products

In this experiment, we assessed peptide formation and degradation of Met during incubation alone or with eight different starting compounds. Analysis of the reaction products showed that Met_2 was created under all nine experimental conditions, both in the presence and absence of additional compounds (Figs. 1, and 2). In contrast, Met_3, Met_{DKP}, Gly-Met, and Met-Gly were not produced when Met was incubated alone but only when additional compounds were present. The Gly-containing peptides, Gly-Met and Met-Gly, were formed even when Gly was not included among the starting materials. Several additional peaks appeared in the same mass chromatograms as Met_{DKP}, Met_2, and Met_3 (indicated by asterisks in Fig. 1). These peaks most likely represent structural isomers of Met_{DKP}, Met_2, and Met_3 composed partially of D-Met. D-Met was not in the starting materials, but a fraction of L-Met was racemized into D-Met during incubation. Such D-Met was then incorporated into Met_{DKP}, Met_2, and Met_3, forming structural isomers.

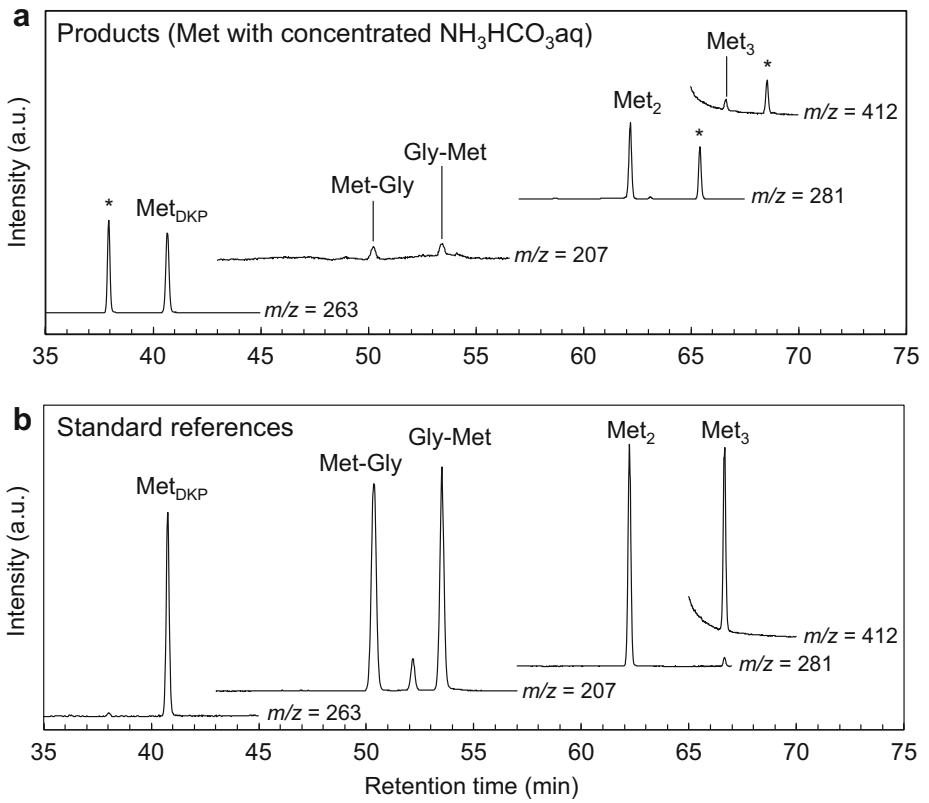

Fig. 1 Mass chromatograms of produced peptides **a** and their standard references **b** from the incubation of Met with concentrated $NH_3HCO_{3(aq)}$ (1.48×10^1 M; 0.43 mmol H_2O) at 175 °C and 150 MPa for 3 h. Peaks marked with asterisks represent structural isomers of assigned peptides partially composed of D-Met. For example, a peak at 37 min would be Met DKP composed of both D-Met and L-Met. Note that the DKP composed of two D-Met molecules is a stereoisomer of the DKP composed of two L-Met molecules. Thus, they appear at the same retention time. However, these homogeneous DKPs are structural isomers of DKP composed of both D-Met and L-Met. Thus, these peaks appear separately

Met Incubation

When pure Met powder was incubated alone, the resulting product was a white powder, even when the reaction was allowed to proceed for 96 h. No weight loss was observed after drying the reaction product (i.e., recovery =100 %), indicating that no volatile compounds were formed during the experiment (Table 1). However, Met yield gradually decreased over the course of the experiment to 60 % by 96 h (Fig. 2a). This indicates that approximately 40 % of Met was converted into other non-volatile compounds. Such non-volatile compounds should contain sulfur, but identifying further details of non-volatile compounds is challenging.

Fig. 2 Residual amounts of Met and Gly and yields of Met peptides (Met_2, Met_3, Met DKP, glycylmethionine, and methionylglycine) under incubation of Met **a** alone, **b** with Gly, **c** with 0.43 mmol H_2O, **d** with 4.3 mmol H_2O, **e** with diluted $NH_{3(aq)}$ (1.48×10^{-1} M; 0.43 mmol H_2O), **f** with concentrated $NH_{3(aq)}$ (1.48×10^1 M; 0.43 mmol H_2O), **g** with diluted $NH_4HCO_{3(aq)}$ (1.48×10^{-1} M; 0.43 mmol H_2O), **h** with concentrated $NH_4HCO_{3(aq)}$ (1.48×10^1 M; 0.43 mmol H_2O), and **i** with $NaOH_{(aq)}$ ▶

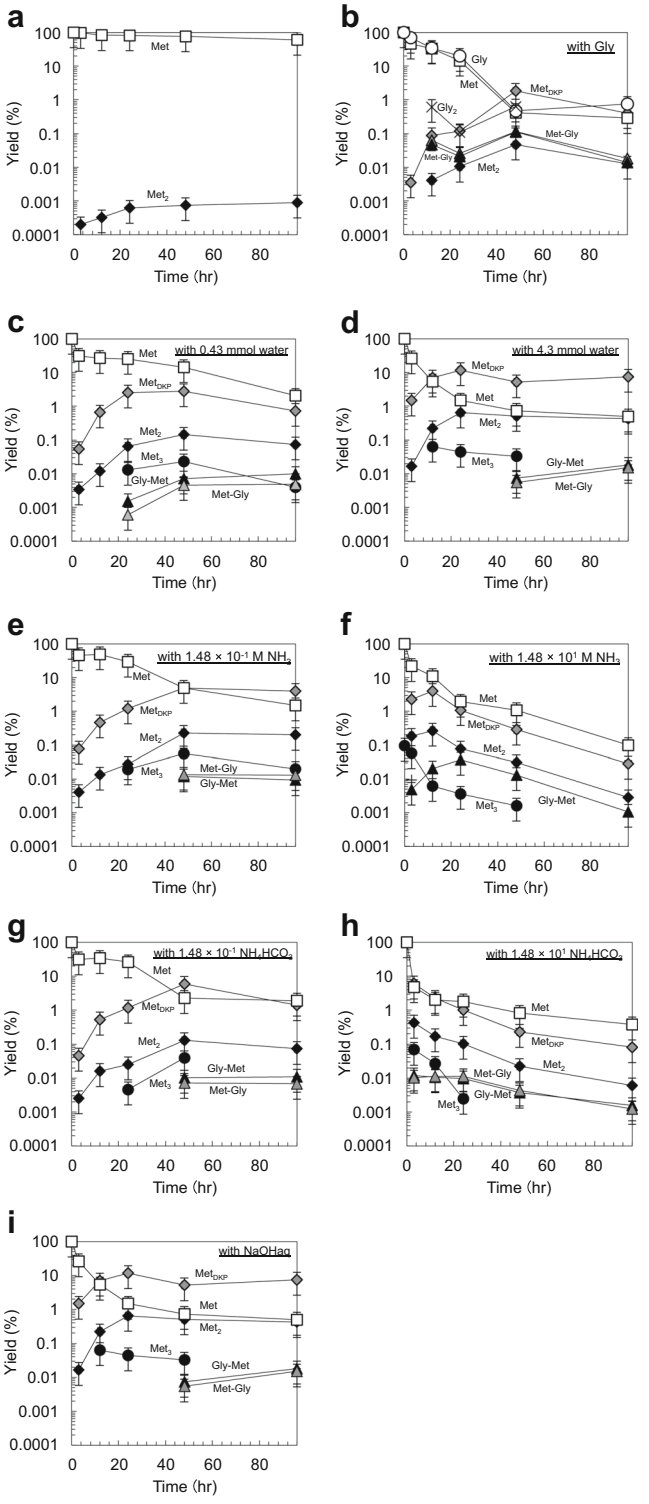
a
Met
Met2
Yield (%)
Time (hr)
b
with Gly
Gly
Met
MetDKP
Gly2
Met-Gly
Met2
c
with 0.43 mmol water
Met
MetDKP
Met2
Met3
Gly-Met
Met-Gly
d
with 4.3 mmol water
MetDKP
Met
Met2
Met3
Gly-Met
Met-Gly
e
with 1.48 × 10-1 M NH3
Met
MetDKP
Met2
Met3
Met-Gly
Gly-Met
f
with 1.48 × 101 M NH3
Met
MetDKP
Met2
Met3
Gly-Met
g
with 1.48 × 10-1 NH4HCO3
Met
MetDKP
Met2
Met3
Gly-Met
Met-Gly
h
with 1.48 × 101 NH4HCO3
Met
MetDKP
Met2
Met-Gly
Gly-Met
Met3
i
with NaOHaq
MetDKP
Met
Met2
Met3
Gly-Met
Met-Gly

Table 1 Recovery of solid residues after experiments and lyophilization

Starting materials	Incubation time (h)	Recovery (%)
Met	96	100
	48	100
	24	100
	12	100
	3	100
Met with H_2O (0.43 mmol)	96	67
	48	82
	24	92
	12	97
	3	111
Met with NH_3aq (1.48×10^{-1} M)	96	77
	48	85
	24	91
	12	100
	3	107
Met with NH_3aq (1.48×10^{1} M)	96	89
	48	87
	24	93
	12	101
	3	100
Met with NH_4HCO_3aq (1.48×10^{-1} M)	96	82
	48	90
	24	94
	12	98
	3	108
Met with NH_4HCO_3aq (1.48×10^{1} M)	96	90
	48	96
	24	101
	12	104
	3	113

Peptides were exclusively composed of Met_2 and were detectable after 3 h (at 0.001 mol%), after which point the yield remained constant up to 96 h.

Met-H_2O Incubation

Water was added to the methionine powder in two experiments: one including 0.43 mmol H_2O and one including 4.3 mmol of H_2O. As the Met powder was not completely dissolved, the water was saturated in terms of Met in both experiments. The product color was pale green after a short duration and brown after 96 h in both experiments.

In both Met-H_2O experiments, Met_2, Met_3, Met_{DKP}, Gly-Met, and Met-Gly were produced (Fig. 2c and d). In the experiment with 0.43 mmol H_2O, recovered Met yield decreased over time to 2.1 mol% at 96 h (Fig. 2c). The yields of Met_2, Met_{DKP}, and Met_3 increased over the first 48 h

and then stayed constant up to 96 h. Gly-Met and Met-Gly were detected after 24 h, even though glycine was not present in the starting materials. The yields of these two peptides increased with elapsed time. In the experiment with 4.3 mmol H_2O, Met yield decreased more rapidly than in the experiment with less water (Fig. 2d), whereas the produced peptides reached higher yields. Peptide yields in this experiment also reached a steady state earlier than those in the prior experiment.

Met-$NH_{3(aq)}$ Incubation

In the two experiments containing added ammonia (1.48×10^{-1} M or 1.48×10^{1} M $NH_{3(aq)}$), the products turned brown by 96 h. The solid residues recovered from the diluted and concentrated ammonia experiments represented approximately 77 and 89 wt%, respectively, of that of the initial Met (Table 1). This was higher than the yield of the Met-H_2O experiment (67 wt% of initial Met).

Experiments with the diluted ammonia (1.48×10^{-1} M $NH_{3(aq)}$ in 0.43 mmol H_2O) provided similar results to those with the identical amount of pure water (0.43 mmol H_2O; Fig. 2c and e). However, the concentrated ammonia solution (1.48×10^{1} M in 0.43 mmol H_2O) had very different effects (Fig. 2c and f). A high initial concentration of ammonia resulted in a more rapid decrease in Met yield (Fig. 2f). Further, with the high ammonia concentration, peptides exhibited the maximum yields after a reduced incubation time. For example, the yields of both Met_2 and Met_{DKP} reached their maximum values at 3 h, which is 45 h earlier than experiments with the diluted ammonia solution. These yields then decreased rapidly until the 96-h point, when they were almost 100 times lower than the maximum yields.

Met-$NH_4HCO_{3(aq)}$ Incubation

In the cases in which 1.48×10^{-1} M or 1.48×10^{1} M $NH_4HCO_{3(aq)}$ were incubated with Met, the final product was brown at 96 h. Yields of Met and Met peptides with the diluted NH_4HCO_3 solution (1.48×10^{-1} M) were similar to those with diluted $NH_{4(aq)}$ (1.48×10^{-1} M). In contrast, the yields of Met and Met peptides with the concentrated $NH_4HCO_{3(aq)}$ were higher than those with the concentrated $NH_{3(aq)}$ (1.48×10^{1} M) (Fig. 2e–h).

Met-$NaOH_{(aq)}$ Incubation

The pH values at room temperature (pH_{RT}) of the 1.48×10^{-1} M and 1.48×10^{1} M $NH_{3(aq)}$ solutions were 11.7 and 13.7, respectively, while the pH_{RT} values of the 1.48×10^{-1} M and 1.48×10^{1} M $NH_4HCO_{3(aq)}$ solutions were 9.1 and 11.5, respectively. To investigate the effect of pH itself on the oligomerization and decomposition of Met, therefore, NaOH was dissolved in 0.43 mmol H_2O to adjust the pH_{RT} of the NaOH solution to 13.7. In the NaOH experiment, the product color changed to brown after 96 h of incubation. A decreasing yield of Met over time was found similar to that observed when an identical amount of water (0.43 mmol) was used (Fig. 2c and i). Compared with the results of the experiment with concentrated ammonia solution, the addition of NaOH led to a similar oligomerization rate but a significantly reduced degradation rate of Met peptides.

Met-Gly Incubation

When both Met and Gly were dissolved in 20 mL water, the pH_{RT} was neutral ($pH_{RT} = 7.1$). After 96 h of incubation of dry mixed powder of Met and Gly, the pH_{RT} of the solution in

which the incubation product was dissolved in 20 mL water was still neutral, although the white powder of the Met-Gly mixture had been converted into a brown tar.

The yield of residual Met decreased slowly over the first 24 h (Fig. 2b), similar to the results of the experiment with 0.43 mmol H_2O (Fig. 2c). However, the degradation of Met subsequently accelerated, so that the yield of recovered Met after 96 h of incubation with Gly was less than half of that with 0.43 mmol H_2O and was more similar to experiments with concentrated $NH_{3(aq)}$ and concentrated $NH_4HCO_{3(aq)}$ (Fig. 2b, f, and h). Yields of Met_2 and Met_{DKP} were similar to those observed in the experiments with diluted $NH_{3(aq)}$ and diluted $NH_4HCO_{3(aq)}$. The Gly-containing peptides Met-Gly and Gly_2 exhibited higher yields than in all other experiments in which Gly was not included in the starting materials.

Sulfur Isotope Compositions

During the incubation of Met alone or of Met and Gly together, the sulfur isotope ratios of the products were not significantly different from that of the starting Met (Fig. 3). In contrast, in the experiments in which H_2O or $NH_{3(aq)}$ were added, the products were depleted in ^{34}S by as much as 2 ‰ relative to that of the starting Met. This isotope fractionation occurred along with decomposition of Met (Fig. 3a) to 2 % of the original amount, even though the solid residues contained more than 60 % of the sulfur originally present in the starting material (Fig. 3b).

Discussion

Effect of Water on Met Oligomerization and Degradation

The presence of water thermodynamically suppresses the formation of peptides due to hydrolysis. However, the incubation of Met with H_2O resulted in greater peptide yields than achieved with Met alone, both at 3 and 96 h (Fig. 4). Peptides are formed from amino acids through an S_N2 reaction that is initiated by the nucleophilic attack of an amino nitrogen on a carbonyl carbon followed by removal of a hydroxyl group. The nucleophilic attack of the S_N2 reaction should occur more frequently in solution due to the capacity for attacking a specific intramolecular geometry. Furthermore, the removal of the hydroxyl in the second step of the reaction requires a proton to form an H_2O molecule. This explains why increasing the amount of water in the starting material resulted in greater peptide yields in our experiments. Even with the greater amount of water (4.3 mmol), the powdered Met was not completely dissolved. Thus, the additional water did not dilute the concentration of Met in the solution but increased the amount of solution that was saturated in terms of Met in the sample. This is likely the reason that peptide yields did not decrease with additional water but rather more peptides were formed more rapidly. We expect that if we had used a much larger amount of water, resulting in an unsaturated Met solution, the peptide yields may have indeed been lower.

The addition of water to the starting materials also affected the degradation rate of Met. The solid residue that resulted from the incubation of pure Met for 96 h contained more than 90 % of the initial sulfur and approximately 60 % of the initial Met (Table 1 and Fig. 3a, b). In contrast, the solid residue that resulted from the incubation of Met with H_2O for 96 h retained approximately 70 % of the initial sulfur and only 2 % of the initial Met (Table 1 and Fig. 3a, b).

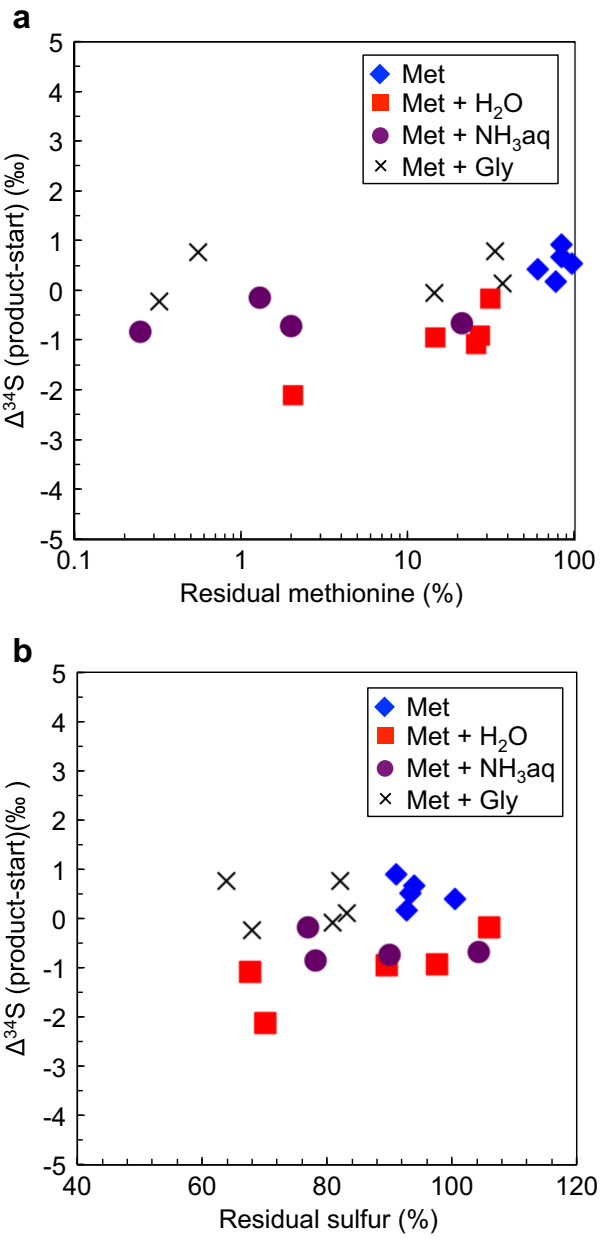

Fig. 3 Stable sulfur isotope ratios of dry residues after experiments. The isotope ratios are shown as $\Delta^{34}S$ values, representing the difference in $\delta^{34}S$ between products and initial Met: **a** $\Delta^{34}S$ value vs. the fraction of the remaining Met in the products and **b** $\Delta^{34}S$ value vs. the fraction of sulfur in the solid residues

Effect of Ammonia and Bicarbonate on Met Oligomerization and Degradation

Figure 4 shows the yields of Met_2 after 3 h and 96 h of incubation with different additives. Because the yield of Met_2 increased over the first 12–48 h and then decreased before 96 h (Fig. 2), the yield at 3 h reflects the rate of Met_2 formation, whereas the difference in yield between 3 h and 96 h reflects the rate of Met_2 degradation (Fig. 4). The only exception to this

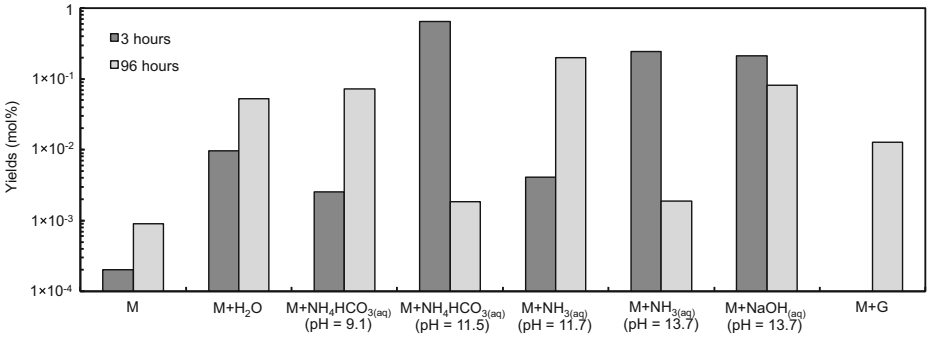

Fig. 4 Comparison of Met_2 yields after 3 and 96 h of incubation. The pH levels shown represent the pH values of solutions added to Met, not the pH values of the initial solutions

is the experiment with concentrated $NH_4HCO_{3(aq)}$, in which the formation and/or degradation occurred so rapidly that the Met_2 yield had already begun to decrease by 3 h (Fig. 3h).

The fact that the Met_2 yield at three hours was higher in the experiment with concentrated $NH_{3(aq)}$ than in the experiment with pure Met indicates that Met_2 formation was promoted by the presence of NH_3 (Fig. 4). Promotion of Met_2 formation was also observed when Met was incubated with the NaOH solution. Therefore, we propose that high pH conditions (pH_{RT} = 13.7) were responsible for the promotion of Met_2 formation. The speciation of Met changes from a zwitterion to an anion when the pH of a Met solution changes from neutral to alkaline. This change would increase the dimerization rate of Met, increasing the nucleophilicity of the N in the amino group. Increased peptide formation of Gly was also reported in a previous study that investigated the oligomerization of Gly in ammonia solution (Oró and Guidry 1961). A similar pH-dependent rate increase was also observed in previous studies that investigated the dimerization rates of Gly in different pH solutions (Rodriguez-Garcia et al. 2015; Sakata et al. 2010). In contrast, negligible differences in the Met_2 formation rate were observed between the experiment with diluted $NH_{3(aq)}$ (pH_{RT} = 11.7) and the experiment with an identical amount of pure water (Fig. 4). Because the amount of ammonia was significantly smaller than that of Met, the pH increase due to the ammonia may have been buffered by Met, resulting in a negligible effect on the rate of oligomerization.

In contrast, a much lower yield of Met_2 was observed at 96 h in the experiment with concentrated $NH_{3(aq)}$ relative to that of the NaOH solution, indicating that this increased degradation of Met_2 was not simply due to the pH increase. The rapid degradation of Met_2 may have been caused by the amidation of Met. This reaction consumes Met and Met oligomers but does not decompose Met into volatile compounds. The presence of this reaction is supported by results that show an increase in the amount of non-volatile components, which is indicated by the higher recovery of nonvolatile compounds in the experiment with $NH_{3(aq)}$ compared to the experiment with H_2O alone (Table 1).

In the experiment with diluted $NH_4HCO_{3(aq)}$, the effects were negligible probably owing to its low molar amount compared with that of Met. When comparing the yield of Met_2 after 3 h of incubation with concentrated $NH_4HCO_{3(aq)}$ or an identical concentration of $NH_{3(aq)}$, Met_2 yield was higher in the incubation with $NH_4HCO_{3(aq)}$ (Fig. 4). This suggests that the presence of carbonate may increase the rate of Met_2 formation. This rate increase cannot be attributed to pH because the pH_{RT} of the concentrated $NH_4HCO_{3(aq)}$ was 11.7, lower than that of the concentrated $NH_{3(aq)}$, 13.7. On the other hand, the yield of Met_2 at 96 h was comparable

between the experiments with concentrated $NH_4HCO_{3(aq)}$ and concentrated $NH_{3(aq)}$ (Fig. 4). This indicates that carbonate does not have a significant effect on the degradation of Met_2.

Effects of Gly on Met Oligomerization and Degradation

When Gly is gradually degraded during incubation, several compounds are produced, including water, ammonia, and carbon dioxide, as well as other products (Cox and Seward 2007). This reaction occurs more rapidly than the degradation of Met observed in this study (Ohara et al. 2007). When Gly was added to the starting materials, the rate of Met oligomerization was initially as slow as that observed with pure Met (Fig. 4). However, the oligomerization rate later increased, and the yield of Met_2 was greater with Gly addition than with pure Met alone after 12 h (Fig. 2a and b). As discussed in sections 4.1–4.3, the presence of water and potentially bicarbonate increases the Met oligomerization rate. Therefore, the increased oligomerization rate observed in the presence of Gly may be attributed to the products of Gly decomposition, particularly water and bicarbonate. Greater yields of peptides composed of both Gly and Met were produced with Gly addition than in experiments with other additives. The rate of peptide formation would also be accelerated by the presence of the degradation products of Gly.

The degradation rate of Met was also greater in the experiment with Gly compared to that found with pure Met (Fig. 2a and b). This elevated Met degradation may also be due to a product of Gly degradation. Degradation rate was also high when $NH_{3(aq)}$ was used as an additive, indicating that ammonia produced during Gly pyrolysis likely affected the rate of Met degradation.

Transition in Sulfur Isotope Ratio

Sulfur isotope compositions of methionine will fractionate when they produce different sulfur species. Direction of sulfur isotope fractionation (i.e., ^{32}S-enrichment or ^{34}S-enrichment) would be dependent on which S-bearing species were produced. In other words, sulfur isotope compositions of products can indirectly constrain the characteristics of S-bearing products. No significant sulfur isotope fractionation was observed in the pure Met and Met + Gly experiments. On the other hand, we found that ^{34}S was depleted in the Met + $NH_{3(aq)}$ and Met + H_2O experiments, by as much as 1 ‰ and 2 ‰ in $\delta^{34}S$ value, respectively (Fig. 3). This may suggest that water was involved in the processes that promoted sulfur isotope fractionation. When water was used in the incubation, a large difference was observed between the fraction of sulfur remaining in the solid residues (70 %) and the yield of residual Met (2 %, Fig. 3). This indicates that almost all of the starting Met was converted into non-volatile sulfur compounds.

Thermal cracking of Met predominantly produces carbon dioxide and/or methanethiol and is the major process of Met decomposition under dry conditions (Yablokov et al. 2009). Degradation of methanethiol produces several S-bearing compounds, including dimethyl sulfide, dimethyl disulfide, H_2S, and elemental sulfur (Coope and Bryce 1954; Mukoyama et al. 2015). Elemental sulfur remains after drying because it is not volatile at the experimental temperature. Isotope fractionation between elemental sulfur and H_2S (and probably also methanethiol, as well) leads to enrichment of ^{32}S in elemental sulfur (Ohmoto and Goldhaber 1997). Therefore, the formation of elemental sulfur is consistent with the enrichment of ^{32}S in the dried residues of our experiments. This further suggests that water promotes

oxidative decomposition of Met. This oxidative reaction competes with oligomerization of Met, owing to the consumption of Met and Met peptides.

Implications for Diagenesis in Prebiotic Earth

In this study, Met peptides as large as trimers (Met_2, Met_{DKP}, and Met_3) were synthesized from monomeric Met powder in the presence of water, ammonia water, and ammonium bicarbonate solution. To the best of our knowledge, no previous study has produced Met peptides larger than trimers while simulating the environmental conditions of early Earth. In comparing the effects of these compounds, we demonstrated that the oligomerization rate of Met is promoted by the presence of water and potentially bicarbonate, as well as high-pH conditions. Thus, these conditions may have played a role in amino acid oligomerization on prebiotic Earth.

Once dissolved in the ocean, amino acids would have been introduced into marine sediments by adsorption on minerals and then concentrated by compaction and dehydration through diagenesis. As there are large variations in the water content of sea-floor sediments, in the course of diagenesis, amino acids should be surrounded by varying amounts of water. The experimental conditions assessed in this study provide such examples and indicate that the reaction rates of both peptide formation and Met decomposition under wet conditions are significantly greater than under dry conditions.

The pH values in majority of natural deep sediments are buffered by minerals. Therefore, pH-changes would have been counteracted than the present experiments. Further, we found no effects of a solution of diluted NH_3 (i.e., 1.48×10^{-1} M) and this concentration is significantly higher than prebiotic ocean estimated in a previous study (2×10^{-6} M) (Summers 1999). The decomposition of organic compounds through diagenesis, however, provides additional ammonia in sediments. High ammonia concentrations in sediments have been suggested by the occurrence of ammonia-intercalated phyllosilicate found in 3.8 Ga-metasediments in Isua, Greenland (Honma 1996). Such ammonia-enriched environments have potential to promote peptide formation, although they also promote the consumption of amino acids and peptides by side reactions.

Bicarbonate may have been enriched in sediments through the degradation of other organic compounds like carboxylic acids and amino acids (Sato et al. 2004). The present study suggests that, in such environments, the formation rates of peptides might have been accelerated.

A large difference in the oligomerization rates of Gly and Met may lead to some difficulty in the formation of peptides containing multiple amino acids. However, this study also shows that the pyrolysis products of Gly promote the oligomerization of Met and enable the formation of peptides containing Met. This indicates that even amino acids with sterically bulky side chains participate in oligomerization reactions following activation from decomposition products of reactive amino acids, thus resulting in the formation of oligopeptides composed of multiple amino acids in diagenesis.

Summary

In this study, we investigated the effects of water, ammonia, ammonium bicarbonate, pH, and Gly on Met peptide formation and Met degradation at 175 °C and 150 MPa. The experimental results are summarized as follows. Met peptides as large as trimers were formed from

monomeric Met. Peptides composed of both Met and Gly were produced from a mixture of Met and Gly, as well as from starting materials that did not initially include Gly, indicating the formation of Gly from Met. The rate of peptide formation from pure, dry Met was very low but increased in the presence of water and with increased pH. Ammonia promoted the rate of peptide formation but also promoted the degradation of Met and Met peptides. The addition of Gly to Met increased the reaction rate of Met peptide formation and Met degradation through the creation of decomposition products of Gly.

Acknowledgments This study was supported by JSPS KAKENHI grant numbers 23740402, 24244084, and 15H02144. The authors thank H. Nakazawa for valuable discussion and F. W. Nara and A. Ishida for sulfur isotope analysis.

References

Bernstein MP, Dworkin JP, Sandford SA, Cooper GW, Allamandola LJ (2002) Racemic amino acids from the ultraviolet photolysis of interstellar ice analogues. Nature 416:401–403

Bujdák J, Rode BM (1999) Silica, alumina and clay catalyzed peptide bond formation: enhanced efficiency of alumina catalyst. Orig Life Evol Biosph 29:451–461

Bujdák J, Faybikova K, Eder A, Yongyai Y, Rode BM (1995) Peptide-chain elongation: a possible role of montmorillonite in prebiotic synthesis of protein precursors. Orig Life Evol Biosph 25:431–441

Cleaves HJ, Aubrey AD, Bada JL (2009) An evaluation of the critical parameters for abiotic peptide synthesis in submarine hydrothermal systems. Orig Life Evol Biosph 39:109–126. doi:10.1007/s11084-008-9154-1

Coope JAR, Bryce WA (1954) The thermal decomposition of dimethyl disulphide. Can J Chem 32:768–779. doi:10.1139/v54-097

Cox JS, Seward TM (2007) The reaction kinetics of alanine and glycine under hydrothermal conditions. Geochim Cosmochim Acta 71:2264–2284

Cronin JR, Moore CB (1971) Amino acid analyses of Murchison, Murray, and Allende carbonaceous chondrites. Science 172:1327–1329

Furukawa Y, Sekine T, Oba M, Kakegawa T, Nakazawa H (2009) Biomolecule formation by oceanic impacts on early earth. Nat Geosci 2:62–66. doi:10.1038/ngeo383

Furukawa Y, Otake T, Ishiguro T, Nakazawa H, Kakegawa T (2012) Abiotic formation of valine peptides under conditions of high temperature and high pressure. Orig Life Evol Biosph 42:519–531. doi:10.1007/s11084-012-9295-0

Furukawa Y, Nakazawa H, Sekine T, Kobayashi T, Kakegawa T (2015) Nucleobase and amino acid formation through impacts of meteorites on the early ocean. Earth Planet Sci Lett 429:216–222. doi:10.1016/j.epsl.2015.07.049

Harada K, Fox SW (1964) Thermal synthesis of natural amino acids from postulated primitive terrestrial atmosphere. Nature 201:335–336

Holland HD (1984) The Chemical Evolution of the Atmosphere and Oceans. Princeton University Press, Princeton

Honma H (1996) High ammonium contents in the 3800 Ma Isua supracrustal rocks, central West Greenland. Geochim Cosmochim Acta 60:2173–2178. doi:10.1016/0016-7037(96)00083-X

Hopkins M, Harrison TM, Manning CE (2010) Constraints on Hadean geodynamics from mineral inclusions in >4 Ga zircons. Earth Planet Sci Lett 298:367–376

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

Kasting JF (1993) Earth's early atmosphere. Science 259:920–926

Kawamura K, Nishi T, Sakiyama T (2005) Consecutive elongation of alanine oligopeptides at the second time range under hydrothermal conditions using a microflow reactor system. J Am Chem Soc 127:522–523. doi:10.1021/ja0447917

Lahav N, White D, Chang S (1978) Peptide formation in prebiotic era: thermal condensation of glycine in fluctuating clay environments. Science 201:67–69

Lemke KH, Rosenbauer RJ, Bird DK (2009) Peptide synthesis in early earth hydrothermal systems. Astrobiology 9:141–146. doi:10.1089/ast.2008.0166

Li F, Fitz D, Fraser DG, Rode BM (2008) Methionine peptide formation under primordial earth conditions. J Inorg Biochem 102:1212–1217. doi:10.1016/j.jinorgbio.2007.12.020

Martins Z, Price MC, Goldman N, Sephton MA, Burchell MJ (2013) Shock synthesis of amino acids from impacting cometary and icy planet surface analogues. Nat Geosci 6:1045–1049. doi:10.1038/ngeo1930

Miller SL (1953) A production of amino acids under possible primitive earth conditions. Science 117:528–529

Mukoyama T, Shimoda N, Satokawa S (2015) Catalytic decomposition of methanethiol to hydrogen sulfide over TiO_2. Fuel Process Technol 131:117–124. doi:10.1016/j.fuproc.2014.11.013

Nakazawa H (2008) Origin and evolution of life: endless ordering of the Earth's light elements. In: Okada H, Mawatari SF, Suzuki N, Gautum P (eds) International Symposium on Origin and Evolution of Natural Diversity, Sapporo, 2008. Hokkaido University, Sapporo, pp. 13–19

Ohara S, Kakegawa T, Nakazawa H (2007) Pressure effects on the abiotic polymerization of glycine. Orig Life Evol Biosph 37:215–223. doi:10.1007/s11084-007-9067-4

Ohmoto H, Goldhaber MB (1997) Sulfur and carbon isotope. In: Barnes HL (ed) Geochemistry of Hydrothermal Ore Deposits. 3rd edn. Wiley, pp 517–612

Oró J, Guidry CL (1961) Direct synthesis of polypeptides. Arch Biochem Biophys 93:166–171

Otake T, Taniguchi T, Furukawa Y, Kawamura F, Nakazawa H, Kakegawa T (2011) Stability of amino acids and their oligomerization under high-pressure conditions: implications for prebiotic chemistry. Astrobiology 11:799–813

Ponnamperma C, Woeller F, Flores J, Romiez M, Allen W (1969) Synthesis of organic compounds by action of electric discharges in simulated primitive atmospheres. In: Blaustein BD (ed) Chemical Reactions in Electrical Discharges, vol 80 Washington, DC, pp 280–288

Rode BM (1999) Peptide and the origin of life. Peptides 20:773–786. doi:10.1016/s0196-9781(99)00062-5

Rode BM, Schwendinger MG (1990) Copper-catalyzed amino-acid condensation in water: a simple possible way of prebiotic peptide formation. Orig Life Evol Biosph 20:401–410. doi:10.1007/bf01808134

Rodriguez-Garcia M, Surman AJ, Cooper GJT, Suarez-Marina I, Hosni Z, Lee MP, Cronin L (2015) Formation of oligopeptides in high yield under simple programmable conditions. Nat Commun 6. doi:10.1038/ncomms9385

Sakata K, Kitadai N, Yokoyama T (2010) Effects of pH and temperature on dimerization rate of glycine: evaluation of favorable environmental conditions for chemical evolution of life. Geochim Cosmochim Acta 74:6841–6851. doi:10.1016/j.gca.2010.08.032

Sato N, Quitain AT, Kang K, Daimon H, Fujie K (2004) Reaction kinetics of amino acid decomposition in high-temperature and high-pressure water. Ind Eng Chem Res 43:3217–3222. doi:10.1021/ie020733n

Summers D (1999) Sources and sinks for ammonia and nitrite on the early earth and the reaction of nitrite with ammonia. Orig Life Evol Biosph 29:33–46. doi:10.1023/A:1006517823004

Yablokov VA, Vasina YA, Zelyaev IA, Mitrofanova SV (2009) Kinetics of thermal decomposition of sulfur-containing amino acids. Russ J Gen Chem 79:1141–1145. doi:10.1134/s1070363209060188