Effects of metal ions and pH on the formation and decomposition rates of di- and tri-peptides in aqueous solution

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The recent discovery of active deep-sea hydrothermal systems venting high-pH fluids suggests that the prebiotic chemistry of the hydrothermal systems of early Earth may have been more diverse than previously thought. To determine the most favorable conditions for prebiotic oligomerization, the effects of metal ions (Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , and Cu^{2+}) and pH on the formation and decomposition rates of glycylglycine (GlyGly), glycylglycylglycine (GlyGlyGly), and diketopiperazine (DKP) in aqueous solution were investigated. Glycine (Gly) solutions containing metal ions were heated for 1–74 days at 140°C under various pH conditions and the samples were analyzed by high-performance liquid chromatography. GlyGly and DKP were produced in all samples regardless of the presence or absence of metal ions. GlyGly yields were higher under basic conditions (pH 9.8–9.9) than under acidic or neutral conditions. Moreover, GlyGly yields in the presence of Cu^{2+} and Zn^{2+} were higher than in the absence of Cu^{2+} and Zn^{2+} ; in the presence of metal ions other than Cu^{2+} and Zn^{2+} , GlyGly yields were lower. The dimerization rate constant of Gly (k_1) increased in the presence of Cu^{2+} . GlyGlyGly was only produced in samples with Cu^{2+} and the yield was 5 times higher under basic conditions (pH 9.8) than under acidic (pH 3.4) or neutral (pH 7.1) conditions. However, other metal ions inhibited prebiotic peptide synthesis by catalyzing hydrolysis or chelation with amino acids. These results reflect the high stability of Cu^{2+} complexes with amino acids or peptides in the salt-induced peptide formation reaction, particularly at high pH. Although elongation of oligopeptides was not favored, formation of metal–amino acid or metal–short peptide chelates may have facilitated primitive biological functions and expanded prebiotic reaction fields because of their mobility in the Earth’s early oceans.

Keywords: salt-induced peptide formation, metal ions, copper (II) complex with glycine, hydrothermal system, prebiotic chemistry, origin of life

INTRODUCTION

Deep-sea hydrothermal systems have been suggested as a suitable environment for chemical evolution in the early Earth (e.g., Corliss, 1990; Holm, 1992; Yanagawa and Kobayashi, 1992; Macleod *et al.*, 1994; Russell and Hall, 1997; Russell, 2003; Holm and Andersson, 2005; Martin *et al.*, 2008). In terrestrial hydrothermal systems, seawater percolates into the deep oceanic crust, reacts with the surrounding rocks at high temperatures, and becomes hot, reduced fluid (up to 400°C) (Tivey *et al.*, 1995; Tivey, 2007). Water–rock interactions produce a broad range of pH and redox conditions in seafloor hydrothermal fluids (e.g., at the East Pacific Rise, Lucky Strike hydrothermal field, Rainbow plume, and Trans-Atlantic Geotraverse area), which may have controlled prebiotic chemistry.

Recently, hydrothermal systems erupting basic and

low-temperature fluids have been discovered in the South Chamorro seamount (Mottl *et al.*, 2003) and Lost City hydrothermal field (Kelley *et al.*, 2001). The high pH of the fluids at these sites has been attributed to serpentinization of olivine (Kelley *et al.*, 2001, 2005; Hulme *et al.*, 2010; Wheat *et al.*, 2010). Serpentinization yields H_2 -rich fluids that facilitate synthesis of organic chemicals (Horita and Berndt, 1999; McCollom and Seewald, 2007; McCollom and Bach, 2009). Therefore, basic hydrothermal systems are regarded as suitable environments for the origin of life (Russell, 2003; Holm *et al.*, 2006; Holm and Neubeck, 2009). Based on petrologic analysis of Archean basalts (Shibuya *et al.*, 2010), it was proposed that Archean subseafloor hydrothermal systems were characterized by high-pH fluids. In addition, there have been several studies experimentally demonstrating prebiotic chemistry at high pH (e.g., Huber and Wächtershäuser, 1998; Russell, 2003; Holm *et al.*, 2006; Sakata *et al.*, 2010). For example, in a previous study, we reported maximum dimerization of glycine (Gly) in aqueous solution at 150°C and pH 9.8 (Sakata *et al.*, 2010).

The metal ions produced by hydrothermal alteration

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Fig. 1. HPLC chromatograms before and after the hydrothermal reactions of glycine in aqueous solution at (a–h) pH 2.2–3.4, (i–p) pH 4.5–7.1, and (q–v) pH 9.8–9.9. (a, i, and q) No metal ions present, (b, j, and r) with Ca^{2+} , (c, k, and s) with Mg^{2+} , (d, l, and t) with Zn^{2+} , (e and m) with Fe^{2+} , (f and n) with Mn^{2+} , and (g, h, o, p, u, and v) with Cu^{2+} . Individual salts were also added to the standard solutions to inhibit relative retention time shifts of the components due to salt effects.

of rocks are also important in prebiotic chemistry. Typical hydrothermal systems, including South Chamorro, release fluids enriched in a variety of metal ions, such as Ca^{2+} , K^{+} , Ba^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , and Pb^{2+} (Mottl *et al.*, 2003; Tivey, 2007; Hulme *et al.*, 2010). Although the concentrations of metal ions in the Lost City hydrothermal field are low, this system is enriched in Ca^{2+} (Kelley *et al.*, 2001, 2005). Recently, it has been suggested that fluxes of organometallic complexes of organic carbon and metal ions (e.g., Cu^{2+} , Fe^{2+}) are higher in hydrothermal plumes than previously assumed (Sander and Koschinsky, 2011). Toner *et al.* (2009) have also shown that organic carbon is associated with Fe in hydrother-

mal plume particles. Thus, the previous belief that most metals released from hydrothermal vents were incorporated into minerals is being reassessed. Fe^{2+} and Mn^{2+} were probably more abundant in ancient oceans than in modern oceans (Anbar, 2008). Therefore, interactions between organic molecules and dissolved metal ions may have been more important in prebiotic chemistry, whereas mineral surface catalysis may have been of lesser importance around hydrothermal vents in the early Earth. In fact, most present-day living organisms contain metalloproteins, which may have been derived from interactions between organic molecules and metal ions in the early terrestrial oceans.

Fig. 1. (continued).

Divalent metal ions, particularly Cu^{2+} , catalyze oligomerization of amino acids in acidic and neutral aqueous solutions (e.g., Schwendinger and Rode, 1989; Eder and Rode, 1994; Imai *et al.*, 1999; Rode, 1999; Li *et al.*, 2010). This reaction is called the “salt-induced peptide formation (SIPF)” reaction (Schwendinger and Rode, 1989). The monochlorocuprate complex is assumed to be the reactive species that leads to linking of the two amino acids to a peptide (Rode, 1999). The monochlorocuprate complex is very stable and partially charged, resulting in nucleophilic attack by the amino group of the chelated amino acid at the protonated carboxyl group of the other amino acids (Rode, 1999). On the other hand, it has been reported that other divalent cations such as Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Fe^{2+} , Zn^{2+} , and Cd^{2+} have little effect on polymerization of Gly (Schwendinger and Rode, 1989; Rode and Schwendinger, 1990). Zn^{2+} catalyzes formation and hydrolysis of diketopiperazines (DKP) (Cronin *et al.*, 1971) and hydrolysis of glycylglycine (GlyGly) (Long *et al.*, 1971).

To date, most prebiotic peptide syntheses have been conducted under acidic or neutral conditions to simulate the typical hydrothermal environment. However, based on the recent discovery of basic hydrothermal systems,

there was likely greater chemical diversity in the hydrothermal systems of the early Earth. Therefore, in this study, systematic heating experiments of Gly with various metal ions (Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , and Cu^{2+}) in aqueous solution under a broad range of pH conditions were conducted to determine the most favorable conditions for formation of peptides. Changes in the reaction rate constants of formation and decomposition of dipeptides, tripeptides, and DKP under various conditions were studied with respect to the glycine-metal complexation mechanism.

METHODS

Analytical grade Gly, GlyGly, and GlyGlyGly were obtained from the Peptide Institute. DKP was obtained from Tokyo Chemical Industry. HCl, NaOH, CaCl_2 , ZnCl_2 , MnCl_2 , and FeCl_2 were obtained from Wako Pure Chemical Industries. MgCl_2 and CuCl_2 were obtained from Kanto Chemical.

Solutions having pH 2.1–2.3, pH 4.5–6.0, and pH 9.8–9.9 of 100 mM Gly and 100 mM Gly/5 mM metal chloride (MCl_2 , $\text{M} = \text{Ca}$, Mg , Cu , Fe , Mn and Zn) were prepared, respectively. The pH of the Gly solutions was adjusted to pH 2.1–2.3 and pH 9.8–9.9 with HCl and NaOH, respectively. The initial pH of the Gly and CuCl_2 solutions was 3.4 and NaOH was used to adjust the pH to 7.1 and 9.8, respectively. The pH of the Gly and the metal chloride solutions ranged from 4.5 to 6.0. Each solution (0.5–1.0 ml) was placed in a Pyrex glass tube and subjected to a vacuum; subsequently, the tube was filled with Ar gas and sealed. To prevent contamination, the Pyrex glass tubes were heated at 500°C for 4.5 h before inserting the samples. Finally, the solutions were heated in an electric oven at 140°C from 1 to 74 days. Before and after the experiment, the pH of the solutions was measured at 25°C with a pH meter (B-212; Horiba). The change in pH before and after the experiments was <1.7. In the Gly and FeCl_2 solutions at pH 2.3 and 5.0, respectively, red iron oxides precipitated after heating; more precipitation occurred at pH 2.3 than at pH 5.0.

Each sample was diluted 10 or 20 times and analyzed using a high-performance liquid chromatography (HPLC) system (ICA-2000; TOA DKK) equipped with a Jasco UV-2075 detector at a wavelength of 200 nm. A reversed phase-type HPLC column (Hydrosphere C18; YMC) was used at 37°C. A 10 mM $\text{C}_6\text{H}_{13}\text{SO}_3\text{Na}$ solution with pH 2.5 was used as the eluent and adjusted using H_3PO_4 (Bujdák and Rode, 1999) at a flow rate of 1.0 ml min⁻¹. Gly, GlyGly, GlyGlyGly, and DKP were identified and quantified by comparing the observed peak retention times and peak areas, respectively, with those of standard compounds. The errors in the concentrations of Gly, GlyGly, GlyGlyGly, and DKP were estimated to be <3.0%.

Table 1. Yields of GlyGly, DKP, and GlyGlyGly; rate constants (k_1 and k_{-1}); and equilibrium constant (E_1) for solutions without and with metal ions (Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , and Cu^{2+}) under various pH conditions and heating times at 140°C

	Heating time (days)	GlyGly (%)	DKP (%)	GlyGlyGly (%)	k_1 ($\text{l mol}^{-1} \text{s}^{-1}$)	k_{-1} (s^{-1})	E_1 (mol l^{-1})
No metal ions (pH 2.3)	74	0.17	0.03	—	6.2×10^{-8}	3.8×10^{-6}	6.1×10^4
Ca^{2+} (pH 2.3)	14	0.15	0.05	—	5.5×10^{-8}	3.7×10^{-6}	6.8×10^4
Mg^{2+} (pH 2.3)	14	0.18	0.07	—	4.9×10^{-8}	2.8×10^{-6}	5.7×10^4
Zn^{2+} (pH 2.3)	14	0.14	0.04	—	6.4×10^{-8}	5.3×10^{-6}	8.2×10^4
Fe^{2+} (pH 2.3)	36	0.15	0.04	—	7.2×10^{-8}	4.7×10^{-6}	6.6×10^4
Mn^{2+} (pH 2.2)	37	0.17	0.06	—	4.1×10^{-8}	2.7×10^{-6}	6.6×10^4
Cu^{2+} (pH 3.4)	45	0.06	0.11	0.01	6.6×10^{-8}	9.3×10^{-6}	1.9×10^5
No metal ions (pH 6.0)	42	0.10	0.10	—	4.3×10^{-8}	4.0×10^{-6}	1.1×10^5
Ca^{2+} (pH 5.7)	15	0.07	0.08	—	1.7×10^{-8}	2.4×10^{-6}	1.4×10^5
Mg^{2+} (pH 5.7)	14	0.05	0.07	—	2.9×10^{-8}	6.1×10^{-6}	2.1×10^5
Zn^{2+} (pH 4.5)	14	0.05	0.07	—	3.7×10^{-8}	7.9×10^{-6}	2.2×10^5
Fe^{2+} (pH 5.0)	30	0.12	0.11	—	2.0×10^{-8}	1.9×10^{-6}	9.4×10^4
Mn^{2+} (pH 5.7)	43	0.06	0.09	—	1.4×10^{-8}	2.5×10^{-6}	1.8×10^5
Cu^{2+} (pH 7.1)	21	0.09	0.09	0.01	5.5×10^{-8}	7.1×10^{-6}	1.2×10^5
No metal ions (pH 9.8)	74	0.62	0.01	—	8.3×10^{-7}	1.4×10^{-5}	1.7×10^4
Ca^{2+} (pH 9.8)	3	0.45	—	—	6.2×10^{-7}	1.4×10^{-5}	2.3×10^4
Mg^{2+} (pH 9.9)	3	0.48	0.001	—	6.2×10^{-7}	1.4×10^{-5}	2.3×10^4
Zn^{2+} (pH 9.8)	37	0.68	0.014	—	4.8×10^{-7}	7.3×10^{-6}	1.5×10^4
Cu^{2+} (pH 9.8)	74	0.62	0.004	0.05	9.7×10^{-7}	1.7×10^{-5}	1.7×10^4

RESULTS AND DISCUSSION

Yields of GlyGly, DKP, and GlyGlyGly

Figure 1 shows the chromatograms of the products obtained from the solutions after the hydrothermal reactions at 140°C under the respective conditions. GlyGly and DKP were produced under all conditions (Fig. 1), while GlyGlyGly was only produced in solutions containing Cu^{2+} (Figs. 1h, p, and v). The relative abundances of DKP and GlyGly increased with increasing heating time. Table 1 and Fig. 2 summarize the yields of GlyGly, DKP, and GlyGlyGly, which were 0.05–0.68%, 0.001–0.11%, and 0.01–0.05%, respectively (Table 1). In this study, the GlyGly yield (0.06%) from the solution containing Cu^{2+} (pH 3.4) was 4 times lower than the yield (0.25%) reported by Rode and Schwendinger (1990), who heated a solution of 800 mM Gly and 400 mM CuCl_2 (pH 2.5) at 80–90°C for 10 days. In the present study, the GlyGly yield (0.12%) from the solution containing Fe^{2+} (pH 5.0) was 2–4 times higher than those (0.03–0.06%) reported by Rode and Schwendinger (1990), who heated a solution of 900 mM Gly and 500 mM FeCl_2 (pH 5.0) at 75°C for 11 days. GlyGlyGly was not detected in the Rode and Schwendinger (1990) experiments. The observed differences are attributed to differences in the concentration,

time, and temperature used in the experiments, which are factors that Cleaves *et al.* (2009) regard as major limiting parameters for peptide synthesis under deep-sea hydrothermal conditions.

The GlyGly yields from the basic solutions (pH 9.8–9.9) were the highest, regardless of the presence or absence of metal ions (Fig. 2a), 3–10 times higher than those from the acidic and neutral solutions. The GlyGly yields from the highly acidic solutions (pH 2.2–3.4) were 2–3 times higher than those from the mildly acidic and neutral solutions (pH 4.3–7.1), except for solutions containing Fe^{2+} and Cu^{2+} . At pH 9.8–9.9, the GlyGly yields from solutions containing Ca^{2+} and Mg^{2+} were 0.7–0.8 times those without metal ions, whereas the GlyGly yields from solutions containing Zn^{2+} and Cu^{2+} were nearly the same as those without metal ions (Fig. 2a). At pH 4.3–7.1, the GlyGly yields from solutions containing Fe^{2+} and Cu^{2+} were similar to those without metal ions, whereas the GlyGly yields from solutions containing Ca^{2+} , Mg^{2+} , Zn^{2+} , and Mn^{2+} were nearly half those from solutions without metal ions (Fig. 2a). At pH 2.2–3.4, the GlyGly yields from solutions containing Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , and Mn^{2+} were similar to those without metal ions, whereas the GlyGly yield from the solution containing Cu^{2+} was 3 times lower than those from solutions without metal

Fig. 2. Comparison of the yields of (a) GlyGly and (b) DKP obtained by heating Gly solutions with or without metal ions under various pH conditions (Table 1). White bars: pH 2.2–3.4, gray bars: pH 4.3–7.1, and black bars: pH 9.8–9.9. Data with error bars on symbols in Fig. 3 do not have error bars in this figure.

ions (Fig. 2a).

In contrast, the DKP yields from basic solutions were much lower than those from acidic and neutral solutions, regardless of the presence or absence of metal ions (Fig. 2b). In most cases, the DKP yields from mildly acidic and neutral solutions (pH 4.3–7.1) were higher than those from highly acidic solutions (pH 2.2–3.4), except for solutions with Mg²⁺ and Cu²⁺. At pH 2.2–3.4, the DKP yields from solutions with metal ions were 1.2–3.5 times higher than from solutions without metal ions (Fig. 2b). At pH 4.3–7.1, the DKP yields from solutions with metal ions were slightly lower than from solutions without metal ions (Fig. 2b).

The yield of GlyGlyGly, which was only produced from solutions containing Cu²⁺, was 5 times higher under basic conditions than under acidic or neutral conditions.

Estimated reaction rate constants

Time profiles for the concentrations of Gly, GlyGly, DKP, and GlyGlyGly are shown in Fig. 3. Under all ex-

perimental conditions, the reaction rates of GlyGly and DKP fell in the following order: pH 4.3–7.1 < pH 2.2–3.4 < pH 9.8–9.9. To estimate the reaction rates, we considered the following reactions, where k_n is the reaction rate constant (Fig. 4): 1) formation of GlyGly from Gly (second-order reaction, k_1), 2) formation of DKP from GlyGly (first-order reaction, k_2), 3) hydrolysis of GlyGly to produce Gly (first-order reaction, k_{-1}), 4) hydrolysis of DKP to produce GlyGly (first-order reaction, k_{-2}) (Qian *et al.*, 1993; Li and Brill, 2003), 5) formation of GlyGlyGly from Gly and GlyGly (second-order reaction, k_3), and 6) hydrolysis of GlyGlyGly to produce Gly and GlyGly (first-order reaction, k_{-3}).

Other than for Cu²⁺, the rate equations for these reactions can be written as:

$$\frac{d}{dt}[\text{Gly}] = 2k_{-1}[\text{GlyGly}] - 2k_1[\text{Gly}]^2 \quad (1)$$

$$\frac{d}{dt}[\text{GlyGly}] = k_1[\text{Gly}]^2 + k_{-2}[\text{DKP}] - (k_{-1} + k_2)[\text{GlyGly}] \quad (2)$$

$$\frac{d}{dt}[\text{DKP}] = k_2[\text{GlyGly}] - k_{-2}[\text{DKP}]. \quad (3)$$

At equilibrium, the rate equations for these reactions can be expressed as a function of the equilibrium constant E_n :

$$k_{-1} = k_1 \frac{[\text{Gly}]_{\text{equilibrium}}^2}{[\text{GlyGly}]_{\text{equilibrium}}} = E_1 k_1 \quad (4)$$

$$k_{-2} = k_2 \frac{[\text{GlyGly}]_{\text{equilibrium}}}{[\text{DKP}]_{\text{equilibrium}}} = E_2 k_2. \quad (5)$$

Equations (1), (2), and (3) can be rewritten using E_1 and E_2 as follows:

$$\frac{d}{dt}[\text{Gly}] = 2E_1 k_1 [\text{GlyGly}] - 2k_1 [\text{Gly}]^2 \quad (6)$$

$$\frac{d}{dt}[\text{GlyGly}] = k_1 [\text{Gly}]^2 + E_2 k_2 [\text{DKP}] - (E_1 k_1 + k_2) [\text{GlyGly}] \quad (7)$$

$$\frac{d}{dt}[\text{DKP}] = k_2 [\text{GlyGly}] - E_2 k_2 [\text{DKP}]. \quad (8)$$

Fig. 3. Time profiles for Gly, GlyGly, and DKP concentrations in the solutions during heating at 140°C. (a) No metal ions present, (b) with Ca^{2+} , (c) with Mg^{2+} , (d) with Zn^{2+} , (e) with Fe^{2+} , (f) with Mn^{2+} , and (g) with Cu^{2+} . White circles: pH 2.2–3.4, gray circles: pH 4.3–7.1, and black circles: pH 9.8–9.9. Solid lines represent fitted peaks used to determine the rate constants.

Similarly, for the Gly and Cu^{2+} solution, the rate equations are as follows:

$$\frac{d}{dt}[\text{GlyGly}] = k_1[\text{Gly}]^2 + k_{-2}[\text{DKP}] + k_{-3}[\text{GlyGlyGly}] - (k_{-1} + k_2)[\text{GlyGly}] - k_3[\text{Gly}][\text{GlyGly}] \quad (10)$$

$$\frac{d}{dt}[\text{Gly}] = 2k_{-1}[\text{GlyGly}] + 2k_{-3}[\text{GlyGlyGly}] - 2k_1[\text{Gly}]^2 - k_3[\text{Gly}][\text{GlyGly}]$$

$$-2k_1[\text{Gly}]^2 - k_3[\text{Gly}][\text{GlyGly}]$$

$$(9) \quad \frac{d}{dt}[\text{DKP}] = k_2[\text{GlyGly}] - k_{-2}[\text{DKP}] \quad (11)$$

Fig. 3. (continued).

$$\frac{d}{dt}[\text{GlyGlyGly}] = k_3[\text{Gly}][\text{GlyGly}] - k_{-3}[\text{GlyGlyGly}]. \quad (12)$$

At equilibrium, the rate equations for these reactions can be written as:

$$k_{-2} = k_2 \frac{[\text{GlyGly}]_{\text{equilibrium}}}{[\text{DKP}]_{\text{equilibrium}}} = E_2 k_2$$

$$k_{-3} = k_3 \frac{[\text{Gly}]_{\text{equilibrium}}[\text{GlyGly}]_{\text{equilibrium}}}{[\text{GlyGlyGly}]_{\text{equilibrium}}} = E_3 k_3. \quad (14)$$

Equations (9), (10), and (11) can be rewritten using E_2 and E_3 as follows:

$$(13) \quad \frac{d}{dt}[\text{Gly}] = 2k_{-1}[\text{GlyGly}] + E_3 k_3 [\text{GlyGlyGly}] - 2k_1[\text{Gly}]^2 - k_3[\text{Gly}][\text{GlyGly}] \quad (15)$$

Fig. 4. Reaction pathways between Gly, GlyGly, GlyGlyGly, and DKP. The reaction rate constants (k_n) are as follows: dimerization of Gly (k_1), hydrolysis of GlyGly (k_{-1}), formation of DKP (k_2), hydrolysis of DKP (k_{-2}), formation of GlyGlyGly (k_3), and hydrolysis of GlyGlyGly (k_{-3}).

$$\frac{d}{dt}[\text{GlyGly}] = k_1[\text{Gly}]^2 + E_2k_2[\text{DKP}] + E_3k_3[\text{GlyGlyGly}] - (k_{-1} + k_2)[\text{GlyGly}] - k_3[\text{Gly}][\text{GlyGly}] \quad (16)$$

$$\frac{d}{dt}[\text{DKP}] = k_2[\text{GlyGly}] - E_2k_2[\text{DKP}] \quad (17)$$

$$\frac{d}{dt}[\text{GlyGlyGly}] = k_3[\text{Gly}][\text{GlyGly}] - E_3k_3[\text{GlyGlyGly}] \quad (18)$$

Equations (6)–(8) or (15)–(18) were fitted to the experimental results to estimate the rate constants using the least squares method and the numerical software Scilab (Fig. 3). Chelation species were not considered in the calculations; the dominant fitting parameters were k_1 and k_{-1} , which were determined within two significant figures.

Table 1 and Fig. 5 summarize the reaction rates (k_1 and k_{-1}) under the various conditions. The dimerization rate constants for Gly (k_1) at pH 9.8–9.9 were one order of magnitude higher than those under acidic and neutral conditions, regardless of the presence or absence of metal ions (Table 1). This is consistent with the conclusion of Sakata *et al.* (2010) that the dimerization rate of Gly is highest at pH 9.8, because Gly zwitterions and anions are present in equal molar fractions (Fig. 6). Generally, at similar pH, the k_1 values were lower for reactions with metal ions than for those without metal ions (Fig. 5a). However, the rate constants for reactions with Cu^{2+} (pH 9.8) were 1.2 times higher than for those without metal ions (Fig. 5a). The hydrolysis rate constants for GlyGly (k_{-1}) were similar regardless of presence and kinds of metal ions (Table 1).

Stability of the Cu^{2+} complex and the SIPF reaction

Eder and Rode (1994) proposed a scheme for catalysis of the SIPF reaction by CuCl_2 , in which the hydrated CuCl_2 binds one amino acid in the chelated form as well as a second amino acid in its protonated form through the

carbonyl oxygen. In this complex, the amino acids facilitate nucleophilic attack of the chelated amino group nitrogen to the carboxyl carbon to form peptides (Eder and Rode, 1994). The Cu^{2+} complex is very stable compared to Ca^{2+} , Mg^{2+} , and Zn^{2+} complexes, as verified by Gibbs free energies, the distances between metal ions and the carbonyl oxygen of Gly, and ionic radii (Remko and Rode, 2006). Thus, the higher k_1 values for Cu^{2+} observed under basic and neutral conditions in the present study can be explained by the higher stability of the Cu^{2+} complex with Gly in the SIPF reaction. The Zn^{2+} complex is the second most stable, resulting in relatively high yields of GlyGly (Fig. 2a).

The lower k_1 values under acidic and neutral conditions can be explained by differences in chemical speciation of Gly under different pH conditions (Fig. 6; Sakata *et al.*, 2010). Dissociation of Gly in aqueous solutions is independent of temperature (Sakata *et al.*, 2010). For chelation of the Cu^{2+} complex with Gly to occur, a carboxylate anion and a lone pair of an amino nitrogen of one Gly molecule must bind with Cu^{2+} . Basic conditions are ideal for chelation, because the anionic state of Gly ($\text{Gly}^-: \text{NH}_2\text{-CH}_2\text{-COO}^-$) is present as well as the zwitterionic state ($\text{Gly}^\pm: \text{NH}_3^+\text{-CH}_2\text{-COO}^-$). Thus, the value of k_1 under basic conditions in the presence of Cu^{2+} is very high. Although under neutral conditions chelation is possible because $\text{Gly}^\pm$ binds with Cu^{2+} , it is less favored because of the lack of Gly^- . However, chelation is difficult under acidic conditions, because Gly is positively charged ($\text{Gly}^+: \text{NH}_3^+\text{-CH}_2\text{-COOH}$). Therefore, the k_1 value under acidic conditions in the presence of Cu^{2+} was similar to that in the absence of metal ions.

Similarly, formation of GlyGlyGly in the presence of Cu^{2+} appears to be related to the steric structure of Cu^{2+} complexes and GlyGly (Kaneda and Martell, 1975; Gergely and Nagypál, 1976; Kittl and Rode, 1981; Eder and Rode, 1994; Remko and Rode, 2006). Figure 7 shows the molar fractions of the seven chemical species of GlyGly-Cu^{2+} complexes with pH (Kittl and Rode, 1981). Kittl and Rode (1981) determined the formation constants for GlyGly-Cu^{2+} complexes using potentiometric titration. The concentrations of Cu^{2+} and GlyGly were 1 and 5 mM, respectively, and the systems were titrated with a 50 mM NaOH solution. All investigations were carried out under nitrogen atmosphere at 20°C and an ionic strength of 200 mM KCl. The systems contained all theoretically possible 1:1 and 1:2 complexes between Cu^{2+} and GlyGly. A Fortran computer program was used to calculate the formation constants. At pH > 7.5, the most abundant species was a Cu^{2+} complex with two GlyGly, with which another Gly molecule could easily react to yield GlyGlyGly. It has been proposed that NaCl also promotes polymerization of amino acids in the presence of Cu^{2+} and promotes formation of di- and tri-peptides

Fig. 5. Comparison of the rate constants (a) k_1 and (b) k_{-1} for solutions with and without metal ions under various pH conditions.

(Eder and Rode, 1994; Rode and Schwendinger, 1990). Therefore, yields of di- and tri-peptides may be higher under seafloor conditions because of high NaCl concentrations.

Hydrothermal fluids are highly reduced and mainly contain Cu^+ species. Sander and Koschinsky (2011) indicated that dissolved copper species bind as Cu^{2+} to organic compounds in hydrothermal fluids once the redox conditions become oxidic, because of mixing of hydrothermal fluids and seawater. Cu^+ may be less active in forming complexes with amino acids than Cu^{2+} , because the electrons in Cu^+ occupy all of the d orbitals, unlike in Cu^{2+} . However, Leal and van den Berg (1998) proposed that Cu^+ forms strong complexes with thiols such as glutathione. Cu^+ may also effectively polymerize amino acids by forming complexes with amino acids. Mixing of hydrothermal fluids and seawater inevitably occurs; therefore, it is important to consider the effect of Cu^{2+} .

Inhibition of peptide formation by metal ions

In the present study, most metal ions, other than Cu^{2+} and Zn^{2+} in basic solutions, decreased dimerization rates and Gly and GlyGly yields (Figs. 2 and 5a). In the solutions containing Fe^{2+} that precipitated red iron oxides after heating, oxidation of iron may have affected the Gly reactions. Apparently, most metal ions promote hydrolysis of peptides rather than synthesis. These results are consistent with those of previous studies (Cronin *et al.*, 1971).

In addition, metal ions may inhibit peptide synthesis

Fig. 6. Molar fractions of dissociation states of Gly as a function of pH at 25 and 140°C. Molar fractions were determined based on published dissociation constants (Sakata *et al.*, 2010; based on Marshall and Franck, 1981; Goldberg *et al.*, 2002; Clarke *et al.*, 2005).

by chelation with Gly and GlyGly molecules. The reduced formation of DKP in the presence of metal ions is attributed to stabilization by chelation with GlyGly (Cronin *et al.*, 1971; Kittl and Rode, 1981; Rainer and Rode, 1982). In metal–GlyGly complexes, the amino or carboxylic group is bound to the metal ion; however, nucleophilic attack to form DKP is inhibited, although DKP has C=O bonds that can coordinate with metal ions. Complexes between DKP and metal ions are positively charged regardless of pH, because DKP does not dissociate in aque-

Fig. 7. Molar fractions of Cu^{2+} -GlyGly chelates as a function of pH at a metal-peptide ratio of 1:5 at 20 °C. "L" indicates a GlyGly ligand (adapted from Kittl and Rode, 1981).

ous solution. Therefore, hydration of DKP by H^+ is not favored under acidic (pH 2.2–3.4) or mildly acidic/neutral (pH 4.3–7.1) conditions because of Coulomb repulsion between the complexes and H^+ , consistent with our results (Fig. 2). Consequently, prebiotic elongation of peptides is not expected under the experimental conditions of the present study. Our conclusion that deep-sea hydrothermal systems are not favorable environments for even simple peptides is in good agreement with Cleaves *et al.* (2009). Most previous studies have focused on the degree of elongation of the peptide bonds (e.g., Lahav *et al.*, 1978; Lawless and Levi, 1979; Rode, 1999; Bujdák and Rode, 2003). However, it is unclear whether elongation of peptide bonds is critical to origin-of-life reactions (Cleaves *et al.*, 2009). Nonetheless, it has been proposed that GlyGly promotes polymerization of amino acids (Plankensteiner *et al.*, 2002). Metal-amino acid or metal-short peptide complexes have various functions, such as self-organization (e.g., Ohata *et al.*, 1995, 1996), self-recombination (e.g., Mizutani *et al.*, 1998), and catalysis (e.g., Weckhuysen *et al.*, 1996) that could contribute to origin-of-life reactions. In addition, these complexes have high mobility in the oceans without undergoing precipitation of metallic ions as minerals (e.g., Toner *et al.*, 2009; Sander and Koschinsky, 2011) and may have facilitated primitive biological functions in the Earth's early oceans.

CONCLUSIONS

In this study, rates of formation and decomposition of GlyGly, DKP, and GlyGlyGly were estimated by heating aqueous solutions of glycine at 140 °C in the presence of metal ions (Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , and Cu^{2+}) under various pH conditions. High-pH aqueous solutions with Cu^{2+} were found to be the most favorable for prebiotic peptide formation; GlyGlyGly was only produced under these conditions. This observation expands the scope of chemical evolution to new types of deep-sea hydrothermal environments such as the Lost City or South Chamorro sea mount that erupt low-temperature, high-pH fluids due to serpentinization. On the other hand, the other metal ions we examined promoted hydrolysis rather than oligomerization of peptides. The various metal ions have different effects because of the relative stability of the complexes (Irving-Williams series) and pH-dependent dissociation of the chelating amino acid ligands. These results do not support the traditional chemical evolution hypothesis that oligomerization of amino acids led to synthesis of proto-proteins (long peptides); however, formation of short peptides is sufficient to achieve biofunctionality, the next step in prebiotic chemistry. Finally, metal complexes between Cu^{2+} and amino acids or short peptides may have contributed to enzyme catalysis in the early oceans because of higher metal ion concentrations.

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