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Effect of temperature on the dissolution and thermal alteration of combined amino acids fixed in natural sediment under simulated hydrothermal conditions

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ABSTRACT

Seafloor sediment containing biogenic amino acids was heated with NaCl solutions at 50–200 °C for 240 h to investigate the dissolution process of amino acids and evaluate their stabilities under hydrothermal conditions. Dissolved amino acids in the combined phase (dissolved combined amino acids, DCAAs) and free phase (dissolved free amino acids, DFAAs) were rapidly released into the solution during heating. The amount of DCAAs in the solutions was 4–9 times higher than the amount of DFAAs at each temperature. When heated at ⩽ 100 °C, most of the total dissolved hydrolyzable amino acids (TDHAAs) were in the combined form (DCAAs/TDHAAs ratios > 0.9). The compositions of the DCAAs in solutions heated at ⩽ 100 °C were similar to that of the total hydrolyzable amino acids (THAAs) of the initial sediment, indicating that the DCAAs, which are derived from organisms and biodebris in the sediment, are barely altered during the hydrothermal reaction at these temperatures. On the other hand, the DCAAs/TDHAAs ratios were 0.72 and 0.57 at 150 and 200 °C, respectively, and the compositions of the DCAAs at 150 and 200 °C were significantly different from that of the initial THAAs. In addition, non-protein amino acids (β-alanine and γ-aminobutyric acid), which are sensitive biochemical indicators of the diagenetic alteration of natural organic matter, drastically increased to 80.9% of the DCAAs after heating at 200 °C. These results suggests that DCAAs are thermally unstable in the hydrothermal solutions at ⩾ 150 °C. These DCAA would be transformed into thermally stable geo-polymers such as humic-like substances and hydrolyzable kerogens.

1. Introduction

Amino acids are essential organic compounds for life and an important source of nitrogen (Fuhrman and Ferguson, 1986; Amend and Shock, 2001; Ogawa and Tanoue, 2003). Dissolved amino acids, peptides, and proteins account for a significant portion of the dissolved organic nitrogen (DON) pool in the ocean (Sigleo et al., 1983; Sommerville and Preston, 2001) and play an important role in the biochemical nitrogen cycle (Lee and Bada, 1977; Keil and Kirchman, 1999). Some studies have revealed that heterotrophic marine bacteria selectively consume dissolved amino acids in the DON (Tupas and Koike, 1990; Jørgensen et al., 1993; Rosenstock and Simon, 1993; Middelboe et al., 1995). It is estimated that amino acids account for 51% of the bacterial organic nitrogen demand (Coffin, 1989). These observations confirm that amino acids are an important nitrogen source for heterotrophic assimilation, and that the concentration and distribution of amino acids are the chemically controlling factors for bacterial growth in the ocean.

Dissolved amino acids consist of a combined phase (dissolved combined amino acids, DCAAs) and a free phase (dissolved free amino acids, DFAAs), and these have been identified at the molecular level in seawater (Ogawa and Tanoue, 2003). The distributions and concentrations of DCAAs and DFAAs have been reported in the case of open sea (Williams, 1986; Gupta and Kawahata, 2003) and coastal areas (Lee and Bada, 1977; Sommerville and Preston, 2001; Kuznetsova and Lee, 2002). Several researchers have found high concentrations of amino acids in high temperature fluids collected from seafloor hydrothermal fields. Hydrothermal fluids over 300 °C collected from the Suiyo Seamount contained 246–1163 nM total dissolved hydrolyzable amino acids (TDHAAs) (Horiuchi et al., 2004). Hydrothermal fluids (42–82 °C) collected from Vulcano Island in Italy contained 3–114 times higher TDHAAs than the

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ambient seawater (Svensson et al., 2004). At the Lost City hydrothermal field located in the Mid-Atlantic Ridge, a higher concentration of TDHAAs was observed in the hydrothermal fluids (47–91 °C, 0.7–2.3 μM) than in the hydrothermal plume (0.6 μM) and ambient seawater (0.3 μM) (Lang et al., 2013). The concentrations of TDHAAs were 4–54 times higher in the high temperature fluids (> 208 °C) than in the low temperature fluids (< 53 °C) and in ambient seawater collected at a hydrothermal field in the Mariana Trough (Fuchida et al., 2014). These findings support the idea that hydrothermal fluids are a pool of amino acids and important media for the supply of organic carbon and nitrogen to hydrothermal ecosystems.

In high temperature fluids, most of the amino acids are in the L-form, and DCAAs and DFAAs are rarely synthesized abiologically (Horiuchi et al., 2004). Biogenic amino acids must be released during the reaction of hydrothermal fluids with sediments, rocks, and chimneys that contain high amounts of amino acids derived from organisms and biodebris (Klevenz et al., 2010; Lang et al., 2013; Fuchida et al., 2014, 2015).

Ito et al. (2006, 2009) heated calcareous ooze with NaCl solution at 100–300 °C to clarify the release process of biogenic amino acids into the solution. They found that amino acids were rapidly released from the sediment heated at 100–150 °C, while they were immediately decomposed at temperatures > 200 °C.

However, the above studies did not clarify the dissolution, decomposition and thermal alteration processes of DCAAs and DFAAs, because they only analyzed the behavior of TDHAAs. Thus, in this study, we observed the effect of temperature on the dissolution and thermal alteration of DCAAs and DFAAs under different hydrothermal conditions between 50 and 200 °C.

2. Experimental

2.1. Materials

The seafloor sediment used in this study was the same sediment whose geochemical features were reported by Fuchida et al. (2015). It was collected from deep seafloor surface (1610 m) of Izena Cauldron, Okinawa Trough (27°14.56′N, 127°03.97′E) during the KT 10–22 cruise (15–21 October 2010, R/V Tanseimaru), and was preserved in a deep freezer (−80 °C) just after sampling. In the laboratory, the frozen sediment sample was dried in vacuum, powdered manually with an agate mortar and pestle, and sieved with #50 (0.3 mm) mesh. X-ray diffraction photometry (Miniflex RAD-IA, Rigaku, Tokyo, Japan) revealed that the sediment was comprised of abundant quartz, some calcite and a small amount of chlorite (Fuchida et al., 2015). The powdered sediment was stored in a freezer at −20 °C until the heating experiments.

2.2. Heating experiments

Powdered sediment (1.0 g) was reacted with 3.5% NaCl solution (15 ml) in a tightly closed polytetrafluoroethylene (PTFE) vessel (25 cm^3) contained in a stainless steel vessel. The vessel was closed after flushing with argon and kept in a drying oven at 50, 100, 150, or 200 °C for 240 h. After heating, the reactant was transferred into a PTFE centrifuge tube (50 cm^3), and then the solid phase was separated by centrifugation (12,000 rpm for 10 min). The separated solution was filtered through a PTFE membrane filter (0.45 μm). The solid phase was dried in a vacuum and powdered manually with an agate mortar and pestle. These samples were stored under refrigeration (−20 °C) until analysis. Three sets of samples were prepared for each experimental condition.

2.3. Hydrolysis of solution and sediment samples with HCl

TDHAAs in the solutions and total hydrolyzable amino acids (THAAs) in the sediments were determined according to previously published hydrolysis procedures (Kawahata and Ishizuka, 1993; Andersson et al., 2000; Ito et al., 2009). The amount of DCAAs was calculated by subtracting the DFAAs in the unhydrolyzed sample from the TDHAAs.

Approximately 3 ml of the sample solution was placed in a glass ampoule (10 cm^3) with 3 ml of concentrated HCl (12 N) (TDHAAs). Sediment sample (0.5 g) was placed in a glass ampoule with 6 N HCl (4 ml) (THAAs). Air was flushed out with Ar, and the ampoule was sealed and heated in a drying oven at 110 °C for 22 h. After cooling to room temperature, the solution was filtered through a PTFE membrane filter (0.2 μm) using a disposable syringe, and then transferred into a pear-shaped flask. Unreacted HCl in the solution was removed by evaporation under vacuum at 40 °C, and the residue was dissolved in 0.1 N HCl. The concentration of the hydrolyzable amino acids was quantified using high-performance liquid chromatography (HPLC) as described below. The error associated with the hydrolysis procedure was within ± 1%.

2.4. Speciation and quantification of amino acids by HPLC

The TDHAAs and THAAs in the hydrolyzed sample solutions and the DFAAs in the unhydrolyzed solution samples were measured with a high performance liquid chromatograph using postcolumn ortho-phthalaldehyde derivation (OPA method; Benson and Hare, 1975). Fifteen proteinaceous α-amino acids, aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), and arginine (Arg), were separated through a cation exchange resin (Hitachi, #2619PH, 4.0 × 150 mm i.d.). β-Alanine (BALA) and γ-aminobutyric acid (GABA), which are categorized as non-protein amino acids, were also detected in this study. The eluent was a citrate buffer solution (trisodium citrate, citric acid, sodium chloride and ethanol), and the flow rate was 0.4 ml/min at 60 °C. The separated amino acids were derivatized in a boric acid buffer solution containing 6 mM ortho-phthalaldehyde and 2-mercaptoethanol, and the amino acid derivatives were detected by a GL-7453 fluorometric detector (GL Science Inc., Tokyo, Japan) (excitation wavelength 360 nm, emission wavelength 440 nm). The detection limit for each amino acid was 10 nM using this method, and the analytical error of the measurement was within ± 0.5%.

3. Results

3.1. DFAAs and DCAAs in the hydrothermal solution

Fig. 1a and Table 1 show the amounts of DFAAs and DCAAs (μmol) in the solutions reacted at 50–200 °C for 240 h. The amounts of DFAAs were 0.08 ± 0.02 μmol at 50 °C and 0.57 ± 0.01 μmol at 100 °C. At 150 and 200 °C, the amounts of DFAAs were 0.48 ± 0.05 and 0.14 ± 0.01 μmol, respectively. The amount of DCAAs was higher than that of DFAAs at each temperature: 0.74 ± 0.04 μmol at 50 °C, 4.86 ± 0.09 μmol at 100 °C, 1.79 ± 0.03 μmol at 150 °C and 1.07 ± 0.12 μmol at 200 °C.

The compositions (mol%) of the DFAAs and DCAAs in the solutions after heating at different temperatures are shown in Fig. 2a and b. Met and Tyr were not detected. Among the DFAAs, the molar percentages of Glu (24.6%) and Thr (19.2%) were high in the solution heated at 50 °C, but were significantly lower at